ENDOCRINOLOGICAL AND SOCIAL MODERATORS OF EMOTIONAL WELL-BEING DURING PERIMENSTRUAL, PERINATAL AND PERIMENOPAUSAL TRANSITIONS

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PUBLISHED IN: Frontiers in Psychology, Frontiers in Psychiatry, Frontiers in Endocrinology and Frontiers in Medicine

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88974-515-9
DOI 10.3389/978-2-88974-515-9

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ENDOCRINOLOGICAL AND SOCIAL MODERATORS OF EMOTIONAL WELL-BEING DURING PERIMENSTRUAL, PERINATAL AND PERIMENOPAUSAL TRANSITIONS

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Citation: Schweizer-Schubert, S., Ditzen, B., Meltzer-Brody, S., eds. (2022). Endocrinological and Social Moderators of Emotional Well-Being During Perimenstrual, Perinatal and Perimenopausal Transitions.
Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-515-9

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frontiers in Psychology

EDITORIAL
published: 02 February 2022
doi: 10.3389/fpsyg.2021.813291

Editorial: Endocrinological and Social Moderators of Emotional Well-Being During Perimenstrual, Perinatal and Perimenopausal Transitions: What Women Want for Sexual Health and Smooth Hormonal Changes

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Keywords: psychoneuroendocrinology, reproductive mood disorders, depression, anxiety, stress, pregnancy, menopause, menstrual cycle

Editorial on the Research Topic

Endocrinological and Social Moderators of Emotional Well-Being During Perimenstrual, Perinatal and Perimenopausal Transitions

OPEN ACCESS

Edited and reviewed by:
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Specialty section:
This article was submitted to Psychology for Clinical Settings, a section of the journal Frontiers in Psychology

Received: *11 November 2021*
Accepted: *22 December 2021*
Published: *02 February 2022*

Citation:
Schweizer-Schubert S (2022) Editorial: Endocrinological and Social Moderators of Emotional Well-Being During Perimenstrual, Perinatal and Perimenopausal Transitions: What Women Want for Sexual Health and Smooth Hormonal Changes. Front. Psychol. 12:813291. doi: 10.3389/fpsyg.2021.813291

Navigating women's mental health between hormonal and social factors has only lately become a focus of research in our international psychoendocrinology community. Research had long been in men's hands; in female hands it started out stymied by contradicting forces—fear of losing the "gender race" in an honest view of biological differences between genders, most notably significant hormonal fluctuations during women's fertile years, vs. the urgent need to identify social(-interactional) parameters women require for well-being and fulfillment of potential, especially in times of hormonal transitions. Nowadays, gender problems for the average couple typically arrive with children and pressures to juggle motherhood and career. Unjust social-interactional patterns in gender roles could constitute a key factor for the significantly higher prevalence of depressive disorders in women vs. men. Stress resulting from social injustice has always compromised mental health. Women with a stress reactivity marked by trauma, either to themselves or (epi-)genetically transmitted, need our particular attention, and here first and foremost those women choosing motherhood next to a career yet lacking adequate support. Their vulnerability to falling into socially detrimental situations and further health problems may in turn lead them into a health service traditionally labeling them as psychiatrically ill or sexually dysfunctional when they are simply suffering from social-interactional injustice. Historically, mankind could have taken a fairer approach, addressing social-interactional stressors instead of additionally attacking female self-confidence by subjecting them to psychiatry. Lack of support to women has been identified as a highly relevant factor for women's higher prevalence of depression (Müters et al., 2013). Particularly a man's lack of emotional support to his female partner, including empathy (Kazmierczak et al., 2015) or humor as stress-relievers during hormonal transitions, increases female risk for depression. Also, a research agenda for women's mental health identified the following gaps in knowledge in a paper on the outcomes of the International Society of Psychosomatic Obstetrics and Gynecology (ISPOG) conference in The Hague: endometriosis, fertility management for cancer survivors, pregnancy deniers, incongruous childbirth expectations and experiences and care provision following birth trauma (Quinlivan et al., 2020).

Gynecological Psychology is still a relatively young field but has also grown in recent years with the addition of a psychoendocrinological focus. From a social-interactional perspective, women's adaptation to endocrine transition periods have been found related to male partners' hormonal fluctuations, a prominent example being the testosterone decline in men prior to childbirth reducing aggression and enabling their increased social support for their female partners such as sharing childcare and home duties (Saxbe et al., 2016). Findings suggest increased risk for female depression and anxiety when these co-fluctuations are out of sync in a couple.

With our article collection we hope to strengthen a Psychoendocrinological Approach to women's mental health that is historically rooted in the biopsychosocial model (Engel, 1977) as well as the stress vulnerability model (Zubin and Spring, 1977). We highlight hormonal fluctuations, biological vulnerability to stress and actual social stressors as key parameters in female depression, focusing on puberty, menstrual cycle, peripartum, perimenopause, alongside female sexual health aiding stress-release in women. We opted for three foci: the GABA-A receptor, central to stress mechanisms; fluctuating sex hormones and the neurosteroid Allopregnanolone; Oxytocin, a hormone at the heart of social interaction.

Alongside new data we provide a thorough review of the key genetic, neuroendocrinological and social-interactional perspectives on the connection between hormonal fluctuations, stress and mental ill-health. Schweizer-Schubert et al. summarize the current state of research on GABA-A receptor and Allopregnanolone, establishing common denominators of "Reproductive Mood Disorders" (depressive symptoms during the above-mentioned hormonal transitions). Which women experience increased sensitivity to fluctuations in sex steroids (estrogen, progesterone) and stress-related steroids? We integrate both dynamics into the concept of "steroid hormone sensitivity" highlighting the role of psychosocial stressors with the aim of providing a theoretical basis for more integrative endocrinological, social and psychological treatment options and prevention strategies for susceptible women.

From puberty and the start of the menstrual cycle, the prevalence of depression in women rises dramatically. McGuire et al. present data suggesting more advanced pubertal physical development and greater rejection sensitivity predicted higher levels of depressive symptoms later. Walsh et al. indicate the role of trauma finding that early life abuse increases effects of intranasal oxytocin on symptoms of premenstrual dysphoric disorder, highlighting the importance of examining women's social-interactional history for predicting hormonal responses. Leeners et al. investigate the association between estradiol, stress perception and stress-related cognitive performance during the menstrual cycle and fertility treatment. Poromaa et al.'s brain imaging study confirms low levels of Allopregnanolone relate to higher serotonergic binding in the prefrontal cortex, crucial to cognitive performance and top-down emotional regulation and the typical pre-ovulatory increase in female well-being.

Concerning peripartal mental health, Melón et al. provide further insights into the role of neurosteroids *via* preclinical testing of a novel therapeutic compound for postpartum depression. Osborne et al. focus on pregnancy's second trimester Allopregnanolone as predictor of post-partum anxiety, which may precede depression. Kress et al. provide a glimpse into their multi-method cohort study protocol (Dresden Study on Parenting, Work, and Mental Health), contributing to greater understanding of the role of social, work and stress factors in mental and somatic health and its long-term endocrinological and transgenerational correlates within the family.

As to women's perimenopausal mental health in their pilot study Gordon et al. provide support for the hypothesis that perimenopausal estradiol fluctuations increase women's sensitivity to psychosocial stress and vulnerability to depression. Mernone et al. examine sexual health in aging women, showing age and postmenopausal status to be negatively associated with sexual functioning, but also that psychosocial factors such as emotional support are crucial to female sexual well-being. In Dickenson et al. we learn about the role of mindfulness in sexual arousal. They examine a potential oxytocinergic neuroendocrine mechanism underlying the link between mindfulness and sexual arousal by looking at subjective and oxytocinergic responses to mindfulness and correspondingly to sexual arousal.

To conclude this collection of articles, a simple question and potential answers—could the removal of the remaining psychosocial stressors for those women still living in patriarchally tinted structures (once again exemplified by the Covid-19 pandemic driving mainly mothers out of the workforce) be the most obvious first step to reduce the significantly higher prevalence of female depression? Let us revisit depression and anxiety as identifiers for unmet human needs due to social ills. Key solutions could be significantly improved societal responses to the female hormonal transitions framing human reproduction and aging and a clear view on requirements for good female sexual health, a fundamental pillar of stress-release for both sexes. A worldwide behavioral change might be required toward a fair management of biological differences between men and women, the ignoring of which has come to deteriorate female well-being in matching fulfillment from motherhood and career. Instead of supporting pharmacological profits from anti-depressants, benzodiazepines and hormonal treatments we could opt for increasing societal profits by applying these latest psychoendocrinological findings to lift psychosocial interactions between the sexes to the next level of social justice. Translations of research into practice are well under way as evidenced by new psychoendocrinology practices, psychoendocrinology courses in medical and psychological faculties and increasing societal awareness of such scientific insights. More to come!

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

ACKNOWLEDGMENTS

We thank Professor Ditzen, dear Beate, for your invaluable inspiration and support!

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Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

REVIEW
published: 18 January 2021
doi: 10.3389/fmed.2020.479646

Steroid Hormone Sensitivity in Reproductive Mood Disorders: On the Role of the $GABA_A$ Receptor Complex and Stress During Hormonal Transitions

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OPEN ACCESS

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Specialty section:
This article was submitted to Obstetrics and Gynecology, a section of the journal Frontiers in Medicine

Received: *20 June 2019*
Accepted: *20 August 2020*
Published: *18 January 2021*

Citation:
Schweizer-Schubert S, Gordon JL, Eisenlohr-Moul TA, Meltzer-Brody S, Schmalenberger KM, Slopien R, Zietlow A-L, Ehlert U and Ditzen B (2021) Steroid Hormone Sensitivity in Reproductive Mood Disorders: On the Role of the $GABA_A$ Receptor Complex and Stress During Hormonal Transitions. Front. Med. 7:479646. doi: 10.3389/fmed.2020.479646

Women worldwide are two to three times more likely to suffer from depression in their lifetime than are men. Female risk for depressive symptoms is particularly high during the reproductive years between menarche and menopause. The term "Reproductive Mood Disorders" refers to depressive disorders triggered by hormonal fluctuations during reproductive transitions including the perimenarchal phase, the pre-menstrual phase, pregnancy, the peripartum period and the perimenopausal transition.

Here we focus on reproductive mood disorders manifesting in adult life. We propose a research agenda that draws together several reproductive mood disorders and investigates which genetic, endocrinological, neural, and psychosocial factors can explain depressive symptoms during phases of hormonal transitions in women. Based on current research it is assumed that some women experience an increased sensitivity to not only fluctuations in reproductive steroids (estrogen and progesterone), but also stress-related steroids. We integrate both dynamics into the concept of "steroid hormone sensitivity," expanding on the concept of "reproductive hormone sensitivity." We suggest that a differential response of the stress steroid system including corticosteroids, neurosteroids, like allopregnanolone and the GABA-A Receptor complex, as well as a differential (epi)genetic risk in serotonergic and GABAergic signaling, are moderators or mediators between changes in the reproductive steroid system and the physiological, affective, and cognitive outcomes manifesting in reproductive mood disorders. We point to the lack of research on the role of psychosocial factors in increasing a woman's stress level and at some point also the sensitivity of her stress steroid system within the etiology of Reproductive Mood Disorders.

Drawing together the evidence on various reproductive mood disorders we seek to present a basis for the development of more effective pharmacological, social, and

psychological treatment interventions and prevention strategies for women susceptible to these disorders. This could pave the way for new research as well as medical and psychological teaching and practice- such as a new type of Practice for Gynecological Psychoneuroendocrinology- with the aim of working on and ultimately offering more integrative forms of support not yet available to women suffering from depression during hormonal transitions. In medical history women have been left alone with this integrative challenge.

Keywords: depression, stress, allopregnanolone, $GABAR_A$ receptor, premenstrual, perinatal, perimenopausal, peripartal

INTRODUCTION

Women all over the world are two to three times more likely to suffer from depression in their lifetime compared to men (1). This risk is particularly high during the reproductive years between menarche and menopause. This suggests that mood disorders triggered by changes in reproductive steroid hormones during reproductive transitions- including the perimenarchal phase with first-onset depression (2–4), the menstrual cycle, pregnancy, the peripartum period and the menopause transition- may account for an important proportion of this increased risk. Indeed, an estimated 13–19% of reproductive-aged women experience clinically significant premenstrual mood disturbance each month (5, 6); 25% experience significant mood symptoms during or following pregnancy (7) and approximately 45–68% of women experience clinically significant mood symptoms during the menopausal transition (8). While each disorder is unique, it is also believed that increased sensitivity to fluctuations in reproductive steroid hormone levels represent an underlying etiologic process common to all three reproductive phases (9). For this reason, this cluster of mood disorders occurring in the context of reproductive transitions are frequently referred to as "Reproductive Mood Disorders" (RMDs) in academic circles and will be the focus of this review.

We will begin by briefly introducing the key neuroendocrine factors featured in all RMDs and will then proceed in the following sections with a more in depth look at three RMDs occurring in adult life: premenstrual dysphoric disorder (PMDD), peripartum depression (PPD), and perimenopausal depression (PMD). We will also describe reproductive steroid hormone environments that characterize each reproductive transition, and the evidence for the involvement of increased sensitivity to reproductive steroid hormones in the etiology of each disorder, as well as the role of stress-relevant mechanisms like the hypothalamic pituitary adrenal (HPA) axis, neurosteroids and $GABAR_AR$ receptors. Furthermore, we end each section with genetic considerations specific to each RMD as well as neuroimaging findings. As a final step, we seek to draw together the evidence from the various RMDs to outline a common etiology, while also discussing potential differences between them. Building on earlier initiatives to discuss a shared etiology between the RMDs [e.g., (10–12)], we expand the previously established concept of reproductive steroid hormone sensitivity toward the concept of "steroid hormone sensitivity," in order to also integrate the contributions of stress-related steroid hormones and the neurosteroid allopregnanolone (ALLO) at the $GABAR_AR$ receptor, a central switch between the reproductive hormonal system and the HPA Axis. We argue that women suffering from RMDs not only have an abnormal reaction to normal reproductive steroid hormone changes, but also an abnormal reaction to normal stress steroid hormone mechanisms including ALLO signaling. Psychosocial factors will briefly be discussed as a pivotal compound in the role of stress sensitivity in these disorders. Taken together, our perspective on RMDs is conceptualized with the ultimate goal of informing their improved diagnosis and treatment as well as improving the early identification of women who are at the highest risk of RMDs to enable prevention.

KEY NEUROENDOCRINE FACTORS IN REPRODUCTIVE MOOD DISORDERS

Sex differentiation in the prevalence of mood disorders begins with puberty (13). Before adolescence, the rates of depression are similar in girls and boys or even slightly higher in boys (12). However, after menarche, a sharp rise in the prevalence of mood disorders and suicidal behaviors in women can be observed (14, 15). Although perimenarchal mood disorders could clearly be considered a RMD, only limited research is available on the underlying psychoneuroendocrinological mechanisms [see for instance Smith (16)]. However, as will be presented in the Neuroimaging Section The Role of Serotonergic Function in RMDs: Possible Direct and Interactive Pathways below, the findings from this strand of research clearly distinguish pubertal depression from the other adult RMDs. Therefore, we here focus on RMDs manifesting in adult life.

Sensitivity to Changes in Reproductive Steroids

Reproductive steroid hormones including estrogens, progesterone and androgens are regulated by the hypothalamic-pituitary-gonadal (HPG) axis. Briefly, as the initial part of the HPG axis, the hypothalamus releases gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to produce and secrete the gonadotropins- luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH passes the bloodstream and stimulates the release of reproductive steroid

hormones from the gonads followed by a negative feedback loop, as illustrated in **Figure 1**. The HPG axis not only regulates reproductive function, but also other physiological functioning, and influences emotion and cognition [as e.g., reviewed in Gurvich et al. (17)].

The marked increase in mood symptoms during times of reproductive transitions suggests that common underlying reproductive steroid hormone-based mechanisms influence mood symptoms in women. The role of reproductive steroid hormones in regulating the switch into dysfunctional affective states in a susceptible group of women has been well-established by studies showing abnormal symptomatic responses to experimental steroid hormone manipulations in those with PMDD (18), peripartum depression (19), and perimenopausal depression (20), however, the source of this susceptibility remains unknown. After nearly two decades of research devoted to clarifying the etiology of RMDs, there is compelling evidence that these disorders are not characterized by absolute *levels* in reproductive steroid hormones but result from an increased sensitivity to *changes* in reproductive steroid hormones instead [see for instance (10, 11, 19–22)]. Therefore, research on RMDs moves more and more toward a focus on understanding affective responses to *changes* in reproductive steroid hormone levels and the underlying neuroendocrinological and genetic mechanisms underlying these responses (23). Also, the ratio between the reproductive steroid hormones estrogen (E2) and progesterone (P4) has been shown to be crucial in the development of psychiatric disorders (24).

Sensitivity to Changes in Stress-Related Steroid Hormones, Neurosteroids, and the $GABA_A$ Receptor Complex

Psychosocial stress is positively associated with the development and severity of mood disturbances during all phases of reproductive transition (25–29). In line with this, there is consistent research showing dysregulations of the hypothalamic-pituitary-adrenal (HPA) axis in depressive disorders. The effector hormones of the HPA axis (most prominently cortisol) act on glucocorticoid- and mineralocorticoid receptors in the central nervous system and provide *negative* feedback to further regulate HPA axis activation [(30); also see **Figure 1**]. Overall, a hyperexcitable HPA axis (for instance as a result of significant early life stress or trauma) has been found to be a common feature of depression (31, 32).

The γ-aminobutyric acid (GABA) system plays a critical role in inhibiting the HPA axis at the level of the paraventricular nucleus (PVN) of the hypothalamus, with GABA as the dominant inhibitory neurotransmitter (33–35). Neuroactive steroids (NAS) are metabolites of cholesterol or steroidal precursors that constitute potent and rapid allosteric modulators of the $GABAR_A$ Rreceptor (36). Among them, a neuroactive P4 metabolite, the neurosteroid 3α-OH-5αβ-pregnan-20-one, or allopregnanolone (ALLO) is of particular interest in this context (37). ALLO acts as a positive allosteric modulator of the $GABAR_A$ Rreceptor with potent anxiolytic and tranquilizing effects. Animal research demonstrates an HPA axis-dampening effect of ALLO: ALLO administration in rats attenuated stress-induced release of adrenocorticotropic hormone (ACTH) and cortisol; ALLO also attenuates the stimulated release of the corticotropin-releasing hormone (CRH) and prevents hypothalamic CRH gene expression in adrenalectomized rodents [reviewed in Girdler and Klatzkin (38)].

During the fetal and infant period, important changes in gene expression related to intracellular chloride concentration resulting in altered GABAergic tone have been observed. Change in gene expression affecting these mechanisms seems to occur for some women, but not all and it can be argued that this can influence a woman's risk for RMDs (37). During early development, GABA is depolarizing and mostly excitatory due to high $[Cl^-]$. Here it plays a key role by regulating a number of processes including the migration, morphological maturation, and differentiation of neurons (39). GABA mediates Cl(-)dependent inhibitory postsynaptic potentials and alterations in these mechanisms e.g., due to epigenetic changes due to excessive stress as described below are related to GABA's potential implication in the pathogenesis of disorders, RMDs (37).

Apart from such alterations in GABA-activated CL- channels, another aspect that is relevant to a better understanding of a potential mechanism sensitizing some women for RMDs is that certain stem cells continue to be excitatory in adulthood. In relation to this aspect of stem cells it can be said that cognitive functions in adults are modulated by hippocampal neurogenesis which in adult humans occurs exclusively on the level of the dentate gyrus (40). The only product of hippocampal neurogenesis are granule cells as the principal excitatory neurons of the dentate gyrus (41). They provide excitatory input to the pyramidal cells of CA3 (42). The precursor cells from which adult neurogenesis originates receive synaptic input from various other brain regions including dopaminergic fibers from the ventral tegmental area, serotonergic projections from the raphe nuclei, acetylcholinergic input from the septum, and GABAergic connections from local interneurons (43, 44).

It has been argued that genetic or epigenetic modifications of the $GABAR_A$ Rreceptor may contribute to a woman's sensitivity to ALLO and, in turn, her risk for mood disturbances in response to reproductive steroid hormone fluctuations. For instance, stress in childhood or puberty has been argued to modify the expression of the $\alpha R_4 R\beta\delta$ $GABAR_A$ Rreceptor and plasticity on mood and cognition in the face of stress (45). This particular receptor subunit combination is sensitive for ALLO. Further, the biosynthesis of ALLO has been argued to be affected by excessive stress, which in turn compromises cognitive and affective functioning (46). As Locci and Pinna (46) emphasize, stress-induced down-regulation of ALLO biosynthesis and changes in the $GABAR_A$ Rreceptor have been related to disorders like posttraumatic stress disorder (PTSD) and depression. On another note, lower serum ALLO levels were found to be associated with higher serotonergic binding in the prefrontal cortex regulating higher cognitive functions and top-down regulation of emotions, while higher ALLO levels were associated with lower alertness (47). Sundstrom Poromaa et al. suggest that this could explain the higher well-being in the follicular phase of the menstrual cycle. Further, sensitivity (and resulting affective vulnerability) might be related to epigenetic changes in genes determining serotonergic functioning (48). However, there

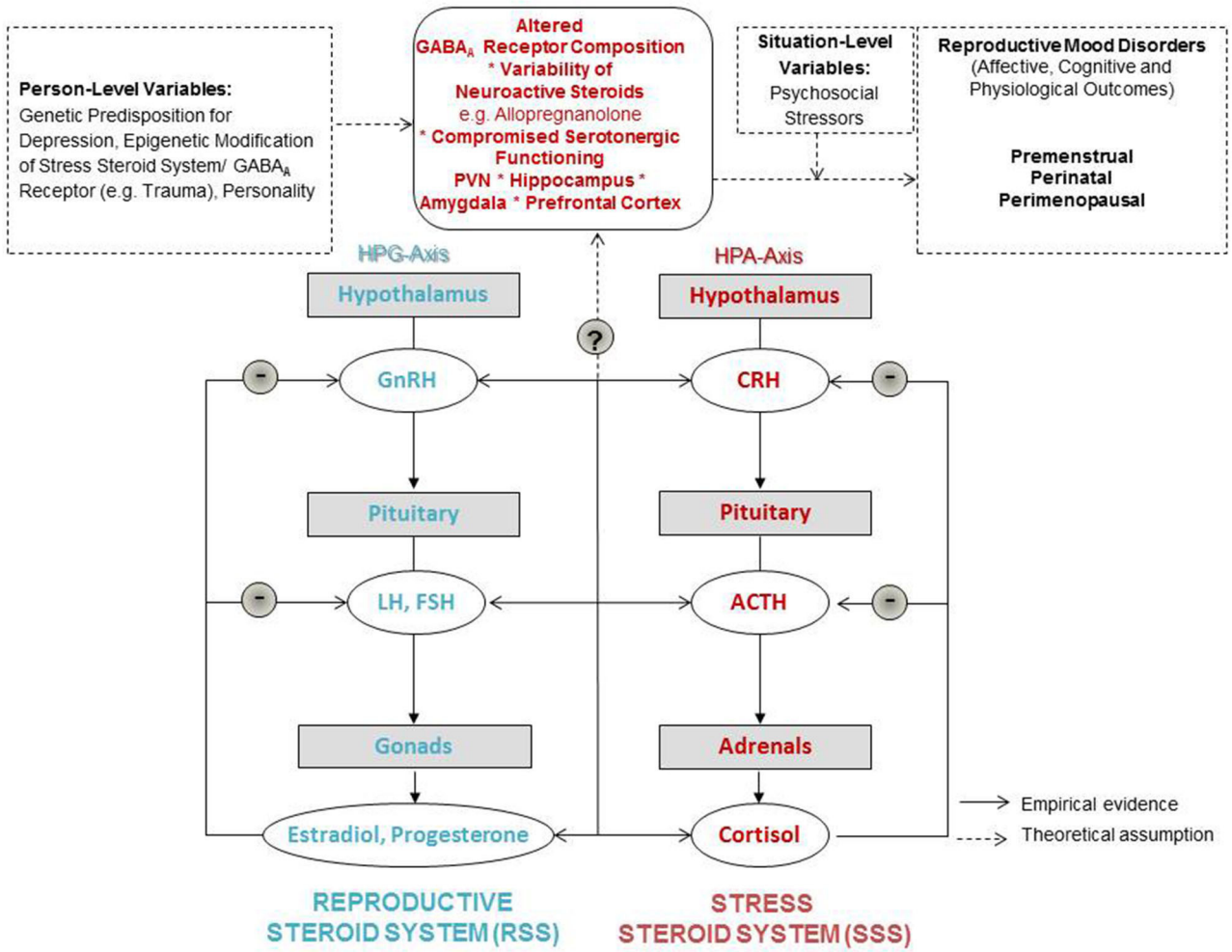

FIGURE 1 | Neuroendocrine modulation of reproductive mood disorders.

is no scientific evidence yet, that abnormalities in serotonergic signaling in depressive responses (49) apply to women suffering from RMDs.

In sum, it is theoretically possible that increased mood sensitivity to ALLO fluctuation (caused by fluctuations in reproductive steroid hormones), manifesting as altered GABAergic tone, would have important consequences for mood outcomes in the face of stress. As such, dysregulation in the stress steroid system is considered in the current manuscript as a potential player in the RMDs, perhaps mediating the relationship between reproductive steroid hormone fluctuation as well as subsequent ALLO fluctuation and mood disturbance in a subset of women. Very recently, a comprehensive review on the mechanisms between GABAergic dysfunction and the HPA axis in the context of depression has been provided (37). However, as it is noted in this review, most of the presented studies have employed male subjects only, and the findings may not be translatable to the female sex. Given the sex differences in stress reactivity (50), behavior (51), and a potential differential in the GABAR$_A$RRsR Rexpression linked to reproductive steroid hormones (52), our review focuses on findings from female subjects.

Concerning the role of ALLO in these mechanisms, at this point, it could be helpful to clearly separate out two issues: First, paradoxical effects of (normal levels of) ALLO due to a epigenetic modification of $\alpha R_4 R\beta\delta$ GABAR and, and second, compromised ability to synthesize ALLO (i.e., low ALLO levels) due to epigenetic modification concerning 3a-HSD and other enzymes needed to synthesize ALLO from P4. They are likely to represent two separate issues, since the first is about sensitivity of the system to normal ALLO changes and the latter is about deficits in levels of ALLO. As will be outlined below, the RMDs described in this manuscript differ in the availability of studies demonstrating the former vs. the latter. In sum, the role of the epigenetically modified GABAR$_A$R receptor composition for the activation of the receptor by neurosteroids needs further elucidation in order to better understand RMDs.

Interactions Between the HPA and the HPG Axes: The Central Role of GABAergic Mechanisms

A link between HPA axis activation and HPG axis suppression has long been established via a suppression of GnRH signaling to the pituitary and corresponding decrease in FSH and LH dampening gonadal activity (53). In depressed females (as well as males) HPA axis activity is inversely related to HPG axis activity (31). Based on this, a bi-directional relationship between the experience of stress and activation of the HPG axis has been suggested: Variability in sex steroids has been related to stress vulnerability and perimenstrual psychiatric risk (24, 54), impaired affective adjustment during the peripartum period (55, 56) and perimenopausal depressive symptoms (20).

At the level of the central nervous system, we consider GABAergic mechanisms as mediators between the HPA and HPG axes in order to advance our understanding of RMDs. The GABAergic Deficit Hypothesis of Major Depression posits that defects in GABAergic neural inhibition can contribute to the etiology of depressive symptoms and that future antidepressant therapies could be improved by focusing on the restoration of GABAergic neurotransmission (57, 58). For comprehensive reviews of the role of the $GABAR_A$ RReceptor, the relevant subunits in mood disorders in the aftermath of stress as well as the role of ALLO and allotetrahydrodeoxycorticosterone (THDOC) in this please see the work of Maguire [e.g., (37)] as well as Locci and Pinna (46).

Both, the HPA and the HPG axes are regulated by GABAergic transmission at the level of CRH and gonadotropin-releasing hormone (GnRH) neurons. Higher neurosteroid sensitivity has been found in $GABAR_A$ RRs incorporating the δ subunit. Therefore, stress-derived neurosteroids at $GABAR_A$ Rreceptors containing δ subunits have been hypothesized to regulate the cross-talk between the HPA and the HPG (59). As suggested from the research above, fluctuations in P4, and subsequent fluctuations in ALLO could be involved in both HPA and the HPG axes functioning. However, evidence on the role of ALLO at central $GABAR_AR$ receptors, their interplay between the HPA and the HPG axes and the genesis of RMDs is very limited, because ALLO mechanisms cannot be measured in the living human brain. Based on the available data, it can be hypothesized that women susceptible to RMDs show higher sensitivity to stress during phases of reproductive steroid hormone variability. This sensitivity corresponds to altered ALLO-dependent functioning at the $GABAR_AR$ receptor in the PVN (see **Figure 1**). In fact, there is evidence of a shift in somatic GABA currents ($ER_{GABA}R$) in parvocellular neurons in the PVN after chronic early life stress (60). In sum, chronic stress seems to lead to compromised GABAergic control of the HPA axis.

Neuroimaging Findings of Reproductive Mood Disorders

Although much of the literature on the neurobiology of MDD, which did not make a distinction between men and women, may still provide valuable insight for RMDs, there are very few functional brain imaging in fact comparing dysfunctional neuronal networks in depressed men and women [e.g., (61)]. Pubertal depression that may manifest as first-onset depression in the perimenarchal phase was found to produce neuroimaging results that significantly differ from the adult Reproductive Mood disorders (PMDD, PPD, PMD) and are more akin to activation patterns observed in Major Depressive Disorder (MDD) (62). The identification of a female depression biotype, as well as differences between female mood disorders across the reproductive cycle can provide essential insights concerning their potential respective treatment. The sub-sections of this article that portray each of these disorders also include a section reviewing the neuroimaging findings for the specific reproductive mood disorder manifesting in adult life.

The Role of Serotonergic Function in RMDs: Possible Direct and Interactive Pathways

Altered serotonergic function has also been observed among women with RMDs, and therefore these mechanisms should be considered when building theoretical and empirical models of RMD. Deficits in serotonergic function are observed across RMDs [as reviewed in Schiller et al. (63)], and pharmacologic fMRI studies have demonstrated that estrogen and progestin administration (vs. placebo) cause increased 5-HT2A binding in brain areas critically involved in emotion regulation, including the anterior cingulate cortex, dorsolateral prefrontal cortex, and lateral orbitofrontal cortex (64). The interactions between sex hormones and the serotonergic system in both healthy women and those with RMDs are extensively reviewed elsewhere [e.g., (63, 65)]. In later sections on various adult reproductive mood disorders, we will discuss both serotonergic abnormalities and potential interactions with the GABAergic system as they relate to specific RMDs.

It could also be the case that the serotonergic and GABAergic systems interact to cause RMD symptoms (66). As reviewed by Birzniece et al. (66), a clear interaction between the GABAergic and the serotonergic systems has been described in the hippocampus. In this particular area of the brain serotonin neurons have been found to frequently end at inhibitory GABAergic interneurons (67). In the context of the relationship between the GABAergic and the serotonergic system in reproductive mood disorders (discussed in more detail below) there have been findings that *in vivo* administration of low doses of a 5HT1A receptor agonist with anxiolytic effects enhances GABA stimulated ClP^- Puptake in cortico-hippocampal synaptoneurosomes (68). More experimental studies are needed to determine the relevance of these interactive mechanisms in the regulation of behavior and RMD symptoms. In this paper, we retain a primary focus on the GABA/ALLO system while acknowledging the importance of the serotonin system in RMDs, as there is a possibility that there are important interactions between the GABAergic and serotonin systems in the etiology of RMDs.

PREMENSTRUAL MOOD DISORDERS

Premenstrual mood disorders include premenstrual syndrome (PMS), PMDD, and premenstrual exacerbation (PME) of ongoing mood disorder (69). It is estimated that 13–19% of naturally cycling women experience clinically significant emotional PMS symptoms (5, 6) and that about 5% meet diagnostic criteria for PMDD (70), a severe form of PMS recently formalized as a new diagnosis in the DSM-5 (71). PMDD is characterized by the recurrent luteal phase emergence of clinically significant affective symptoms that become minimal or absent after the onset of menstruation (72–74). This "on-off" pattern in which symptoms are fully *confined* to the luteal phase has been emphasized as a critical diagnostic feature for selecting "pure" PMS/PMDD (i.e., without comorbidities) in research studies; however, there may be important variability in the exact timing of the onset and resolution of symptoms in PMDD and related syndromes (75). With respect to PME prevalence, prospective data from epidemiologic studies indicate that roughly 60% of women with depressive disorders demonstrate clinically significant PME of at least one depressive symptoms (76). Therefore, premenstrual mood disorders affect a large portion of women. While PMDD was not coded for in the ICD-10, PMDD is new in ICD-11 where it is put under gynecologic disorders, but is cross-listed as a depressive disorder. This might cause confusion, since there is no evidence for a gynecologic pathology in PMDD. ICD-11 will not come into effect until the year 2022, which means that it is currently not used in most places. At the moment there is an ICD-11 Browser made available by the World Health Organization (WHO), where these new categories can be found (World Health Organization, ICD-11 Browser, Version 2019).

Sensitivity to Changes in Reproductive Steroids in PMS/PMDD

The reproductive steroid hormones E2 and P4 change in a predictable fashion across the prototypical 4-week menstrual cycle. In the first week, which starts with menstrual bleeding, both E2 and P4 are low and stable, followed by a large abrupt peak in E2 at the end of the second week, just prior to ovulation. In the third week, formation of the corpus luteum after ovulation leads to a two-week elevation of P4 and secondary elevation of E2. In the final week, both E2 and P4 plateau and then fall precipitously in the final few days prior to menses onset. In the paragraphs that follow, we outline what is known about the pathophysiology of PMS/PMDD. Since the pathophysiology of PME of depression has been found to be unique from PMDD but has yet to be clarified in its own right (i.e., PME of depression is resistant to various effective treatments for PMDD; Bixo et al. (77), Freeman et al. (78), Peters et al. (79)], we will focus our review below on the role of reproductive steroid hormones in PMS/PMDD (i.e., those with luteal phase confinement of symptoms).

As yet, studies have failed to demonstrate consistent abnormalities in E2, P4, or ALLO hormone levels among women with PMS/PMDD. Instead, premenstrual mood disorders are thought to be caused by an aberrant response to normal fluctuations in reproductive steroid hormones. A series of clinical trials demonstrate that GnRH agonists effectively treat the symptoms of PMDD by creating a medical menopause characterized by low, stable levels of reproductive steroid hormones [reviewed in Wyatt et al. (80)]. However, experimental studies demonstrate that addback of luteal phase levels of E2, P4, or their combination causes a resurgence of PMDD-like symptoms not observed in controls and not precipitated by placebo (18). Recently, Schmidt et al. (22) have also demonstrated that this symptomatic response to addback of reproductive steroid hormones is time-limited, remitting after 1 month of stable addback. This series of experiments suggests that it is the postovulatory *changes* in reproductive steroid hormones—and not the elevated luteal hormone levels themselves—that precipitate symptoms in PMS/PMDD.

Further, experimental work demonstrates that PMS/PMDD are caused by an abnormal sensitivity to normal postovulatory *surges* in reproductive steroid hormones rather than perimenstrual reproductive steroid hormone *withdrawal*. Schmidt et al. (81) tested the effects of reproductive steroid hormone withdrawal on symptoms of PMDD in three groups of women; one third received placebo in the midluteal phase, and the other two thirds received mifepristone, a competitive P4 receptor antagonist that causes menses (breakdown of endometrium) and luteolysis (involution of the corpus luteum and associated E2 and P4 withdrawal). Of those receiving mifepristone, half also received human chorionic gonadotropin (HcG), which rescued the corpus luteum, preventing reproductive steroid hormone withdrawal (while still allowing mifepristone-induced menses for blinding purposes); the other half of those on mifepristone received a placebo, which resulted in both mifepristone-induced menses and mifepristone-induced luteolysis with attendant early reproductive steroid hormone withdrawal. The authors reported no significant mood differences between the three groups (midluteal induction of early menses and hormone withdrawal with mifepristone + placebo injection, midluteal induction of early menses but normal midluteal hormone levels with mifepristone + HcG injection, and a natural midluteal phase without menses or hormone withdrawal on dual placebos). This indicates that neither initiation of menses nor induction of hormone (and attendant ALLO) withdrawal alone underlie symptoms of PMDD. In combination with the results of studies described above, we conclude that an abnormal post-ovulatory sensitivity to *surges* in reproductive steroid hormones (and not a perimenstrual sensitivity to reproductive steroid hormone withdrawal) likely precipitates the symptoms of PMS/PMDD.

Sensitivity to Changes in ALLO and the HPA Axis in PMS/PMDD

Although general psychiatric populations have been found to show reduced biosynthesis of ALLO (46), a recent experimental metabolomics study demonstrated normal P4 metabolism to GABAergic neurosteroids (e.g., ALLO) in PMS/PMDD relative to controls (82). Instead of being caused by a general reduction in ALLO levels (e.g., similar to that observed in depression or PTSD), evidence is accumulating to support the notion that

PMS/PMDD are caused by an abnormal neural sensitivity to normal ALLO changes. A recent randomized controlled trial demonstrated preliminary evidence for efficacy of dutasteride in PMDD, a 5α-reductase inhibitor that prevents the metabolism of P4 to its GABAergic neurosteroid metabolites such as ALLO (83). This indicates that it is an altered sensitivity to postovulatory ALLO surges, and not solely E2 or P4 surges, that triggers symptoms of PMS/PMDD.

This altered response to ALLO surges in PMS/PMDD might be caused by abnormal or insufficient plasticity of the $GABAR_AR$ receptor in response to the postovulatory rise in ALLO [e.g., an upregulation of the α4βδ $GABAR_AR$ receptor; Shen et al. (84)]. This argument is supported by preliminary evidence for the efficacy of UC1010 (Sepranolone), which acts as an antagonist at the neurosteroid binding site of the $GABAR_AR$ receptor at which ALLO is active, thereby preventing the adverse effects of ALLO surges in PMDD (77). Notably, these combined results would appear to rule out insufficient ALLO biosynthesis (either peripherally or in the CNS) as the cause of PMS/PMDD, since blockade of the neurosteroid binding site is therapeutic rather than further triggering PMS/PMDD symptoms. In sum, it appears that symptoms of PMS/PMDD are probably related to an abnormal regulation of the $GABAR_AR$ receptor in response to postovulatory surges in ALLO.

Perimenstrual studies demonstrate that GnRH-agonist suppression of reproductive steroid hormones blunts the normative HPA axis response to stress in healthy women, and that addback administration of luteal levels of P4—and not E2—recapitulate the luteal phase potentiation of the HPA axis response to stress (85, 86). Since ALLO is known to limit the extent and duration of the HPA axis response to stress, it is likely that luteal phase increases in P4 potentiate the HPA axis response through other (e.g., genomic) mechanisms (87).

While the HPA axis and related cortisol output has been extensively studied in observational studies of PMS/PMDD, there is no consistent evidence for abnormal cortisol levels or responses to stress in PMS/PMDD [reviewed in Kiesner and Granger (88)]. Rigorous experimental studies (in which women with and without PMDD are tested during ovarian suppression with GnRH agonist, E2 addback, and P4 addback) demonstrate no differences in reproductive steroid hormone regulation of the HPA axis among women with PMS/PMDD compared to controls, including normal effects of administration of reproductive steroid hormones on output of CRH by the hypothalamus, ACTH by the pituitary, and glucocorticoids by the adrenals, as well as normal effects of administration of reproductive steroid hormones on glucocorticoid receptor feedback (85). Therefore, this may be an area of pathophysiology in which PMS/PMDD differs from other mood disorders such as unipolar depression and may also differ from the pathophysiology of peripartum or perimenopausal mood episodes (compare e.g., also sections HPA Axis Dysregulation and the Neurosteroid Hypothesis of Peripartum Depression and HPA Axis and GABA-ergic Mechanisms in Perimenopausal Depression).

Despite normal regulation of the HPA axis by reproductive steroid hormones (85), there is some evidence to indicate that trauma and recent life stressors increase the severity of hormone-related symptom expression in PMS/PMDD. Cross-sectional studies have observed a correlation between traumatic experiences and retrospectively-self-reported PMDD symptoms [a method known to have a very high false positive rate; Pilver et al. (89)]; however more rigorous studies in which PMDD was prospectively-diagnosed did not find increased exposure to trauma in individuals with PMS/PMDD (90). PMDD patients with high ALLO levels have been found to show blunted nocturnal cortisol levels in comparison to healthy controls who had low ALLO serum concentrations. It also has been argued that diurnal secretion of cortisol may be influenced by ALLO levels during the luteal phase (91). However, these findings do not replicate in another experimental study on PMDD, where affected women did not show altered ALLO metabolism following ovarian suppression and E2 or P4 addback (82). Nonetheless, a longitudinal study of patients with prospectively-diagnosed PMDD found that the strength of the daily link between P4 levels and symptoms was stronger in patients with histories of trauma, which may indicate that trauma increases the *severity* (rather than the occurrence) of hormone sensitive mood symptoms in PMS/PMDD (92). In addition to historical trauma exposure, current life stressors may exacerbate or prolong the mood effects of hormone sensitivity in women with PMS/PMDD. In one observational study, cycles preceded by higher-than-usual perceived stress showed greater premenstrual increases in mood symptoms (93). Another prospective study of medical students who were (or were not) beginning a stressful night shift assignment found greater increases in premenstrual mood *changes* among students starting the stressful rotation (94). Since the HPA axis has been found to be normal in PMS/PMDD, more work is needed to understand how internal and environmental stressors may interact with hormone sensitivity to increase cyclical mood symptoms.

Brain Imaging Findings on PMDD

Neuroimaging studies of PMDD remain rare and of mixed quality [for a thorough review and critique of this literature, see Comasco and Sundström-Poromaa (95)]. However, a few patterns of interest have emerged and have begun to shed light on some possible neurobiological underpinnings of PMDD. A few studies comparing gray matter volumes between women with PMDD and healthy controls have observed larger posterior cerebellum and increased gray matter density in the hippocampal cortex, as well as lower gray matter density in the parahippocampal cortex (96, 97). Some studies have noted differences in brain function between those with and without PMDD regardless of cycle phase, including altered activation in the dorsolateral prefrontal cortex (98) and increased amygdala response to negative stimuli (99). Other studies show differences in how the cycle impacts brain function in PMDD. In a proton magnetic resonance spectroscopy study comparing PMDD and controls, cortical GABA decreased from the follicular to the luteal phases in controls, but increased from the follicular to the luteal phase in PMDD (100). Amygdala function may also respond abnormally to progesterone in PMDD; one study found that GABAergic progesterone metabolites predicted lower

amygdala reactivity to social negative pictures in healthy controls, whereas the opposite relationship was found in those with PMDD (99). Finally, one study found that, relative to healthy controls, women with PMDD showed greater late luteal phase increases in activation of the left insula during a cognitive processing task (101). In sum, while there are some promising findings that may point to the underlying neurobiology of hormone sensitivity in PMDD, more systematic, and experimental imaging work is required to move this area forward.

Interactions Between the Serotonergic and the GABAergic system in PMDD

As discussed by Birzniece et al. (66) SSRIs may increase inhibitory processes in the limbic structures of the brain involved in the emotional as well as cognitive regulation by hyperpolarization of neuronal membranes enhancing GABA-stimulated Cl^- uptake. Women diagnosed with PMDD have been shown to have a decreased sensitivity toward $GABA_A$ receptor active substances, especially during the luteal phase (102), when altered serotonergic activity is also observed [reviewed in Hantsoo and Epperson (103)]. Serotonin reuptake inhibitors represent an effective treatment, especially by means of intermittent administration in the luteal phase (104). One possible mechanism is that the SSRI treatment increases metabolism of progesterone to allopregnanolone and normalizes the tolerance to neurosteroids observed during the luteal phase in PMDD (105). However, there is also some evidence that the benefit of SSRIs in PMDD is dependent on their serotonergic mechanisms (106), since coadministration of the serotonin receptor antagonist metergoline (vs. SSRI alone) is able to undermine the benefit of SSRIs in PMDD. Therefore, more work is needed to determine whether alterations of serotonin and GABA systems represent additive or interactive risk factors for PMDD.

Genetic Factors in PMS/PMDD

Although a few studies have identified single-nucleotide polymorphisms (SNP) that differentiate PMS/PMDD cases from controls (107, 108), caution should be exercised when interpreting such studies (109). However, with a focus on epigenetic alterations a recent study of lymphoblastoid cell line cultures from women with PMS/PMDD and controls has demonstrated notable abnormalities in the cellular epigenetic processing of reproductive steroid hormones in PMDD, including altered mRNA expression of several ESC/E(Z) complex genes, both in control samples and in samples treated with E2 and P4 (110). Epigenetic changes across the menstrual cycle and in response to hormonal manipulations may represent a critical area of research to move forward our understanding of the pathophysiology of PMS/PMDD.

PERIPARTUM MOOD DISORDERS

Diagnostic codes for peripartum mood disorders in the ICD and DSM diagnostic manuals differ and there is not a consistent definition. The ICD-10 (111) restricts to either pregnancy or postpartum onset, while in the DSM-5 (71), there is a "peripartum onset" specifier that includes both pregnancy and postpartum in the affective disorders section. ICD-11 distinguishes between mild, moderate, and severe episodes of depression and also between single and recurrent episodes, as well as with and without psychotic symptoms that are supplemented with "current episode perinatal" in the codes ICD 11 6A70-6A71 (World Health Organization ICD-11 Browser, Version 2019).

Up to 25% of women experience significant depressive symptoms following pregnancy (7, 112), however, it is estimated that about 80% of postpartum depression (PPD) cases are not recognized and officially diagnosed, which means that only 20% of affected women receive the treatment they need. Notably, affective symptoms are often already present during pregnancy and the presence of such symptoms in pregnancy has been shown to be among the strongest predictors of PPD (113).

Reproductive Hormonal Changes During the Peripartum Period

For a comprehensive overview concerning normal reproductive steroid hormone fluctuations see Schock et al. (114). It has long been hypothesized that withdrawal of reproductive steroid hormones occurring in the postpartum period plays an important role in the etiology of PPD. One of the first studies providing strong evidence of this used a hormone manipulation paradigm simulating the reproductive steroid hormone withdrawal after parturition among women with a history of PPD and among women without. It was shown that the same hormone manipulation procedure produced a significant increase in depressive symptoms among the women with a history of PPD but not among women without. This suggests that PPD may result from an increased sensitivity to the changes in reproductive steroid hormones that characterize the postpartum period (19). Also, there are several small RCTs finding hormone therapy to be an effective treatment for postpartum depressive symptoms which also supports the role of hormonal withdrawal in the etiology of PPD (115).

While the mechanisms by which withdrawal from E2 and P4 triggers depressive mood in a subset of postpartum women are not fully understood, there is a growing body of research suggesting that increased sensitivity to psychosocial stress may play a role. For example, a history of stressful life events is a strong predictor of the development of PPD (116). Furthermore, psychological (e.g., adjustment to the role as a mother) and physiological stressors (e.g., sleep deprivation) markedly affect subjective well-being, affective symptoms, and physiological stress responses after birth (117). In addition, standard laboratory experiments have shown that stress-susceptibility prior to birth can predict postpartum mood symptoms (118).

HPA Axis Dysregulation and the Neurosteroid Hypothesis of Peripartum Depression

Stress increasing factors for depression and anxiety for instance during pregnancy have been reviewed as risk factors for these disorders, e.g., lack of partner or of social support, a history of

abuse or of domestic violence; personal history of mental illness; unplanned or unwanted pregnancy; adverse events in life and high perceived stress; present and past pregnancy complications and pregnancy loss (119). In light of the important role that psychosocial stressors and increased sensitivity to such stressors likely play in the development of peripartum depression, it is not surprising that there is more and more evidence implicating alterations in HPA axis functioning in the etiology of peripartum depression (119). The cortisol response of pregnant women (and new mothers, respectively), with a vulnerability for depression during reproductive transitions may vary as a function of the social support they receive, as well as other stress reducing interventions they may have access to (120). Pregnant women suffering from depression show a significantly higher cortisol response to stressors compared to their controls (118). Pregnant women with prepartum depression and/or anxiety disorder measured in the third trimester have also been found to show a higher cortisol response to stress, but only in the case of comorbidity with e.g., anxiety disorders (121). Also, higher mid-term CRH levels were associated with PPD (122). Further, a significant correlation between the cortisol awakening response and major depression was found in pregnant women (123). Setting pre-partum and postpartum mood problems into context in peripartum research can contribute to our understanding of RMDs, as we see a continued rise in E2 in the third trimester accompanied by an increase in mood symptoms and ultimately also the peak in depressive symptoms in the second and third postpartum week where accumulated pre-partum psychiatric burden adds up with postpartum hormone withdrawal (124).

In recent years, research on peripartum depression has evolved toward examining the role of neurosteroids (e.g., ALLO and DHEA) in the genesis of the disorder and a "hormone-sensitive" phenotype of postpartum depression has been proposed [e.g., (125)]. Dehydroepiandrosterone (DHEA) plays a particular role in affective dysregulation and abnormal DHEA secretion has been found in major depression [e.g., (126)]. Also, DHEA has been found to have anti-depressive effects on both, men and women (127). However, the neurosteroid ALLO has increasingly moved into the center of attention. Regardless of PPD or a healthy state, postpartum women show reduced cortical GABA and ALLO, comparable to healthy women in their follicular phase (128), which also suggests that normal absolute hormone levels are not the underlying cause, but the fluctuation of hormones. There appears to be a vulnerability or sensitivity in a subpopulation of women to the development of peripartum depression (125). It has been shown that the increasing levels of neuroactive steroids (NAS) during pregnancy are crucially related to modifications in the expression of specific $GABAR_A$ Rreceptor subunits (129). During pregnancy and after parturition, due to major changes in reproductive hormones, the expression of the $GABAR_AR$ receptor δ subunit is altered (52, 130, 131). ALLO levels increase during pregnancy and the $GABAR_A$ Rreceptor δ subunit is downregulated. After parturition ALLO levels decrease rapidly, the $GABAR_A$ R receptor δ subunit is recovered. Based on research with rodents it has been hypothesized that i0n women at risk for PPD, this regulatory mechanism seems to be compromised. For a comprehensive review on these mechanisms, also with respect to the stress axis we recommend the most recent work by Walton and Maguire (132). Altered NAS and GABA profiles have been found in women at risk for peripartum depression. Peripartum GABA levels in women at risk for PPD manifested at a significantly lower level compared to healthy controls (133). In pregnant women whose stress regulatory mechanisms are compromised with respect to this $GABAR_AR$ receptor complex (for genetic or epigenetic reasons), there can be extremely high stress levels. So, the vulnerability to stress-related psychological disorders like anxiety and depression increases significantly, depending also on the potency of exogenous stressors such as social stressors (119). Neurosteroids play a key role in endogenous stress modulation (134). In sum, as it is hypothesized that a flexible plasticity of $GABAR_AR$ receptor subunits is compromised in PPD women, suggesting a dysfunction in adapting to peripartal hormonal changes. This would also correspond with the key notion proposed by Rubinow and Schmidt (23) that RMDs represent a problem of switching between affective states.

Brain Imaging Findings on Peripartum Depression

Women with PPD have been found to show altered functional connectivity and activity in brain areas key for executive functions as well as emotion and reward processing. For a comprehensive review of structural and functional connectivity and molecular imaging research please see Duan et al. (135). Neuroimaging findings on PPD center around a decreased resting state functional connectivity between the anterior cingulate cortex, amygdala, hippocampus, and the dorsolateral prefrontal cortex against the background of falling progesterone and ALLO levels after giving birth (136). First fMRI and spectroscopy studies have started to bring GABA and ALLO mechanisms on screen in peripartum depressed women (137). GABA levels were found to be correlated with marked differences in the connectivity of the dorsomedial prefrontal cortex within the default mode network, which also correlated with depression cores in PPD women (137). Also, compromised connectivity has been reported between the amygdala and prefrontal cortex, which implies dysfunctions in emotion processing (138). Concerning an overlap between MDD and PPD functional abnormalities, findings are conflicting. While some authors argue that the network dysfunctions are identical between the disorders (138, 139), others argue that only perimenarchal depressive disorders share the same neuroimaging findings with MDD as mentioned earlier (62). Here, it has to be considered that the majority of neuroimaging studies had included cases suffering from PPD and MDD as well. Future research will have to make clear distinctions in this respect. In particular the hypothalamus, the amygdala, the anterior cingulate, the orbitofrontal and dorsolateral prefrontal cortices, the insula and the striatum seem to be key areas of interest to the etiology of PPD (140). In sum, fMRI studies demonstrate hypoactivation of brain regions studied in women with postpartum depression compared to those without PPD (62).

Interactions Between the Serotonergic and the GABAergic system in Peripartum Depression

The link between the serotonergic system and the GABAergic system in the peripartum period is a research area of increasing interest. Antidepressant treatment with SSRI's has been shown to restore plasma and CSF ALLO levels in association with improvements in depressive symptoms (141, 142). Therefore, increasing levels of ALLO may play an important role in the antidepressant and anti-anxiety pharmacological effects of SSRIs (46). In addition, the recent FDA approval of brexanolone (Zulresso) for the treatment of postpartum depression provides further evidence that a positive allosteric modulator of GABA-A improves depression in the postpartum period (143, 144). However, the relation between brexanolone treatment in women with PPD and associated serotonin levels has not yet been shown.

Genetic Factors in Peripartum Depression

There is a growing interest in understanding the genetic signature of peripartum depression. The literature consists largely of smaller genetic epidemiological and linkage studies (145–149). More recently, a study of the heritability of PPD using the Swedish national twin register estimated the heritability of PPD to be 54 and 44% using twin and sibling designs, respectively. In this same population, the heritability of depression occurring outside of the peripartum period was estimated to be 32% (150). These findings suggest that one-third of the genetic contribution to peripartum depression was unique and did not overlap with the genetic component of depression outside of the peripartum period. Therefore, the increased heritability, as well as increased genetic overlap with other mood disorders, makes peripartum depression an interesting candidate for future genetic investigations. Genetic aspects represent an important person-level variable in a potential etiological model of reproductive disorders, as will be shown in the graphical abstract introduced below as a precursor to such an etiological model (section Discussion: From "Reproductive Steroid Hormone Sensitivity" to "Steroid Hormone Sensitivity").

Underlying pathophysiology can be used to probe the heterogeneity of peripartum depression. We hypothesize there may be different phenotypes of peripartum depression. Evidence from the international PACT Consortium (Postpartum Depression: Action Toward Causes and Treatment), has worked to help define the heterogeneity of peripartum depression and has described different phenotypes based on severity of symptoms, timing of onset of depressive symptoms, and presence of suicidal thoughts, race, and ethnicity among others (151–153). The PACT Consortium is also interested in the underlying genetic signature of PPD and is currently working on a large-scale Genome-wide association study of PPD (154). These approaches will increase our understanding of phenotypic differences but there remains an important gap between defining phenotypes and understanding the underlying mechanistic differences.

Consequently, the next step is to investigate the underlying pathophysiology that may be unique to the observed heterogeneity in PPD. For example, it could also be hypothesized that women with a susceptibility to fluctuations in reproductive steroid hormones who already show signs of depression during pregnancy may not develop a natural tolerance toward ALLO changes during pregnancy and postpartum, which could imply that they show an increased sensitivity in their key receptors for ALLO. The recent approval of brexanolone, a formulation of ALLO, in the US for PPD is highly relevant to this hypothesis. The women studied in the clinical trials had severe PPD with onset of symptoms in the third trimester or within the first month postpartum (143, 144).

PERIMENOPAUSAL MOOD DISORDERS

The menopausal transition describes the reproductive phase transitioning from regular menstrual cycles to the complete cessation of menses, which marks the onset of menopause. Between ages 42 and 55, nearly all women experience the menopause transition, which, on average, extends 5–6 years leading up to the last menstrual period [e.g., Avis and McKinlay (155), Oldenhave et al. (156), Treloar (157)]. A recent review article identified 12 cross-sectional studies comparing rates of elevated depressive symptoms in pre- and peri-menopausal women and concluded that 45–68% of perimenopausal women, vs. only 28–31% of premenopausal women, report clinically significant elevations in depressive symptoms (8). Currently, perimenopausal depression does not have a diagnostic code that is distinct from major depression in either the ICD-10 (111) or the DSM-5 (71). ICD-11 still does not code for perimenopausal depression- this RMD would have to be coded by "Other specified menopausal and perimenopausal disorders, GA20Y" (World Health Organization, ICD-11 Browser, Version 2019).While the Center for Epidemiologic Studies Depression Scale (CES-D) is the questionnaire that is most commonly used to assess depressive symptoms in the menopause transition, there has been a call for a menopause-specific scale that would better take both physical and affective symptoms into consideration (158). In this review, we focus on reproductive hormonal fluctuations as a critical factor in perimenopausal problems. However, for the sake of completion- and as will also be referred to in the concluding research outlook- other stress responsive systems also need to be taken into consideration. For instance, the immune system, as well as the thyroid hormonal system can be at the root of climacteric symptoms (159). So it is of utmost importance to rule out other causes like thyroid disorder or autoimmune disorder (160) in women presenting with perimenopausal depression. Also, the links between the reproductive hormonal and the immune system have to be kept in mind, in particular in the context of Hormone Therapy in the Perimenopausal Transition (161). Fluctuations in Progesterone levels have also been shown to alter immune responses and susceptibility to infections at diverse mucosal sites including the genital, gastrointestinal, and respiratory tracts. So the immunomodulatory effects of Progesterone-based compounds

need to be given thorough consideration in the treatment of perimenopausal symptoms (162). Reproductive hormonal changes seem to be at the core of those interactions with other hormonal system disorders as well as immunological disturbances, so for this review, we focus on fluctuations in reproductive hormones.

Reproductive Steroid Hormone Changes of the Menopause Transition

The menopausal transition is characterized by several hormonal changes, triggered by a diminishing number of ovarian follicles and fluctuating levels of FSH (163). Beginning in the early menopause transition and continuing into the late transition, is the appearance of menstrual cycles that are characterized by elevated luteal phase E2 levels, which can reach levels that are as high as double those generally seen in the late follicular phase (164, 165). E2 levels in the early follicular phase, on the other hand, have been shown, at times, to reach lower levels than typically observed in reproductive-aged women (166). Furthermore, the low-E2 early follicular phase lengthens due to a delayed ovarian response to FSH, resulting in a longer cycle (167). While P4 levels remain intact throughout the early menopause transition, luteal P4 is lower, on average, throughout the late menopause transition (168). Anovulatory cycles, characterized by low P4 but variable E2 levels, also become increasingly common, with 60–70% of cycles being anovulatory in the late transition (169).

Evidence that an Abnormal Sensitivity to Normal Perimenopausal Reproductive Hormonal Changes Contributes to Perimenopausal Depression

Several studies implicate extreme fluctuation in E2 in the development of perimenopausal depression. Perhaps the strongest evidence comes from a placebo-controlled study by Schmidt et al. (20), which experimentally induced E2 withdrawal using an E2 patch and observed a marked increase in depressive symptoms in the following weeks among women with a history of perimenopausal depression that had been responsive to E2, but not among those without. These findings are in line with three studies observing a relationship between greater E2 fluctuation and elevated risk for depressive symptoms (170–172), as well as a recent placebo-controlled RCT examining the efficacy of transdermal E2 in the prevention of depressive symptoms among perimenopausal and early postmenopausal women (173). In this 12-month study, the rate of clinically significant depressive symptoms was found to be lower among women assigned to transdermal E2 (100 ug/day) vs. placebo (17 vs. 32%). Interestingly, women in the early menopause transition were found to experience the greatest mood benefit, perhaps suggesting that its effects were due to its ability to stabilize the hormonal environment rather than increase low E2 levels.

HPA Axis and GABA-ergic Mechanisms in Perimenopausal Depression

In light of the importance of both hormonal and psychosocial contributions to the development of perimenopausal depression, it has been hypothesized that the hormonal environment of the menopause transition, particularly increased E2 fluctuation, may interact with the stress axis to confer an increased sensitivity to psychosocial stress and increased vulnerability to mood disturbance (163). However, when compared to PMDD and peripartum depression, research directly examining this potential interaction is greatly lacking. In one study cited above (163), a significant interaction was found between E2 fluctuation and the presence of major stressful life events such that E2 fluctuation was predictive of depressive mood among women experiencing one or more events but not among those without baseline stress. Furthermore, the 12-month RCT of transdermal E2 cited above found that the mood benefits of E2 were enhanced among women with greater baseline stressful life events (173). While two studies to date suggest that basal cortisol levels are unrelated to depressive mood in the menopause transition (126, 174), there is some evidence that altered diurnal cortisol patterns may be associated with perimenopausal depressive symptoms. For example, an ancillary of the Study of Women Across the Nation (SWAN) including 408 midlife women, found that depressive symptom score was associated with a flatter diurnal cortisol slope (175). A second study has also observed a relationship between greater weekly changes in E2 and elevated morning cortisol among women with current perimenopausal depression but not among euthymic controls (171, 172). This latter finding would be consistent with the notion that increased HPA axis activation following E2 (and, in turn, ALLO) fluctuations may play a role in perimenopausal depression.

The potential involvement of the GABAergic system and GABAergic neurosteroids, such as ALLO, in the etiology of perimenopausal depression remains largely theoretical as little clinical research directly testing its contribution has been conducted. In a rat model, ovariectomy has been shown to greatly reduce brain ALLO concentrations while administration of 17β estradiol (0.1 or 1 ug per day for 14 days) has been shown to restore ALLO to pre-ovariectomy levels in the hippocampus and hypothalamus (176), an effect that is likely explained by E2's modulation of the enzymes involved in the conversion of P4 to ALLO, 5α-reductase, and 3α-hydroxysteroid dehydrogenase (177). It is therefore possible that the shift between the more extreme E2 levels that characterize the menopause transition, either alone or in conjunction with P4, may result in a more dramatic change in ALLO concentrations than would be seen in a typical menstrual cycle, thus triggering depressive mood in a subset of women for whom the $GABAR_AR$ receptor fails to adjust to the rapid change in neurosteroid levels. These women may also be more prone to depressive mood when first experiencing a hypogonadal state in the late perimenopausal and early postmenopausal phases: in line with this possibility, a recent study examined the correlation between serum ALLO levels and mood in 140 late perimenopausal and early postmenopausal women and found that ALLO was negatively correlated with feelings of guilt among the early postmenopausal women (29).

Brain Imaging Studies in Perimenopausal Depression

To date, there has been little research examining the neural correlates of perimenopausal depression. One study by Berent-Spillson et al. (178) compared brain activation patterns in euthymic pre-, peri-, and postmenopausal women in response to an emotion identification task. Perimenopausal women were found to have a negative bias in identifying the emotions exhibited by the neutral faces; furthermore, both peri- and postmenopausal women exhibited less limbic activation and greater activation of the tempo-parietal-occipital junction (178), a pattern considered to indicate a more cognitively-mediated decision-making process than an emotionally-mediated one. The cognitively-mediated decision-making process was associated with greater depressive symptoms across all three groups. Laboratory sessions occurred in the early follicular (low E2) phase among cycling women, though E2 levels did not correlate with any of the outcomes assessed, suggesting that these differences are not related to differences in hormone *levels*, but that perhaps greater hormonal instability and/ or hormonal withdrawal is the hormonal driver behind these observed differences. Other imaging studies, while not conducted in perimenopausal women *per se*, have used experimental hormonal manipulations in other populations to inform our understanding of the menopause transition. These studies have primarily focused on the involvement of the serotonergic system and are therefore described in the next section.

Interactions Between the Serotonergic and the GABAergic system in Perimenopausal Depression

As with PMDD and PPD, the potential involvement of other neurotransmitter systems apart from GABA have been investigated in perimenopausal depression, with the serotonergic system receiving the most attention. Neocortical serotonin transporter binding (resulting in greater serotonin reuptake and therefore lower serotoninergic tone in this region) has been found to increase in response to an experimentally induced hypogonadal state mimicking that observed in the late menopause transition (49). Furthermore, cortical serotonin transporter binding has been found to decrease (179) while 5-HTR_{2A}R receptor binding has been found to increase (180) following the administration of estrogen therapy in postmenopausal women. One final study examining serotoninergic influences in perimenopausal depression examined the interaction between tryptophan depletion and estradiol treatment on brain activation during an emotion identification task among early postmenopausal women (181). Specifically, it was found that tryptophan depletion reduced activation of the dorsolateral prefrontal cortex and medial frontal/cingulate gyrus compared to sham depletion but that this effect was eliminated with the administration of estradiol. Also relevant to the potential involvement of the serotonergic system in perimenopausal depression is one study comparing the volume of monoamine oxidase A, an enzyme involved in the degradation of serotonin, in the prefrontal cortex of pre-, peri-, and postmenopausal women (182). This study found that on average, women in the perimenopausal age range (41–51 years) had 34% greater monoamine oxidase A volume compared to young reproductive-aged women and 16% greater than older women.

The above studies implicating the serotonergic system in the etiology of perimenopausal depression are consistent with research finding that selective serotonin reuptake inhibitors (SSRIs) are an effective treatment for perimenopausal depression [see Maki et al. (183) for review]. Taken together, these research findings suggest that the serotonergic system has a role to play in the etiology of perimenopausal depression; however, in light of the complex interactions that are well-documented between the GABAergic and serotonergic system, this certainly does not preclude the simultaneous involvement of the GABAergic system as more thoroughly provided above. Interactions between the two systems remain to be explored in depth in the perimenopausal context.

Genetic Factors in Perimenopausal Depression

Current research examining potential genetic contributions to the development of perimenopausal depression is limited. The largest study to date involved 1,538 pre- and perimenopausal women ages 42–55 participating in the Study of Women's Health Across the Nation (SWAN). In this study, specific polymorphisms of three genes involved in the metabolism of E2 and estrone (E1)–the CYP1A1 gene among Caucasian and African American women, the 17HSD gene among Chinese women, and the CYP 19 gene among Japanese women – were found to be associated with an increased risk of elevated depressive symptoms (184). However, neither the ESR1 nor the ESR2 gene, respectively, encoding estrogen receptors α and β, were found to be relevant for risk of depressive symptoms. A second study including 488 women ages 42–68, of which 156 were perimenopausal (54 depressed and 102 controls), observed depression-related differences in the frequency of polymorphisms for the MAO-A and MTHFR genes, which are relevant for monoamine oxidation and methylation, respectively. However, several other genes, including the ESR1 gene, several genes involved in serotonergic transmission (5HTR2A, 5HTR1B, and 5HTR2C), and the GABRB1 gene, which codes for a subunit of the $GABAR_A$R receptor, were not found to be relevant for depression in the perimenopausal subgroup (185). However, statistical power to detect such effects may have been limited. A third study including 391 Chinese women (191 with major depressive disorder, 200 controls) found that, among women ages 40–60, a gene-by-environment interaction between negative life events and allelic variations of the ESR2 gene could be seen in relation to major depressive disorder. However, no such interaction was observed among younger women, raising the possibility that the hormonal environment of the menopause transition, when combined with negative life events, may trigger depressive mood in women with a genetic predisposition involving the ESR2 gene. However, there is clearly much work to be done in clarifying the role that genetics

play in the etiology of perimenopausal depression. Future research that more carefully defines the menopause transition via menstrual bleeding patterns using the Stages of Reproductive Aging Workshop +10 (STRAW+10) criteria (186), excluding postmenopausal women, is needed. The STRAW+10 guidelines define the early menopause transition based on the appearance of a menstrual cycle length 7+ days shorter or longer than usual and the late menopause transition based on the occurrence of two skipped cycles, but <1 year since the last menstrual period.

DISCUSSION: FROM "REPRODUCTIVE STEROID HORMONE SENSITIVITY" TO "STEROID HORMONE SENSITIVITY"

Based on the research findings presented in this review, the following conclusions can be made with a fair degree of confidence. First, rigorous hormonal manipulation studies have demonstrated strong evidence that increased mood sensitivity to changes in reproductive steroid hormones plays a key role in the etiology of all three RMDs discussed—PMS/PMDD (18), PPD (19), and PMD (20). The evidence that increased sensitivity to ALLO fluctuation mediates the link between reproductive steroid hormone changes and mood disturbance is quite strong in the context of PMDD (83) and PPD (143). However, further work is needed to examine the role that sensitivity to ALLO fluctuation triggered by E2 or P4 fluctuation may play in the development of perimenopausal depression. Although it seems likely that genetic and epigenetic contributions play an important role in conferring an increased sensitivity to ALLO fluctuation, we are far from having a clear and consistent picture about the specific genes involved.

A second assertion that can be made at this stage of research on RMDs is that proximal psychosocial stress increases susceptibility for mood disturbance in the context of reproductive transitions. In the case of PPD and perimenopausal depression, there is some evidence that HPA axis dysregulation may either be a correlate of depressive symptoms or may play a partial role in the development of mood disturbance. However, the evidence for its role as a primary mechanism underlying the etiology of these RMDs is relatively weak. More work is needed, in particular also on PMDD, to identify the mechanisms by which psychosocial stress may increase risk for and/or exacerbate RMDs. Overall, the $GABAR_AR$ receptor in specific subunit combinations has been found to be a clear center piece in these mechanisms between the stress hormonal axis and the reproductive hormonal axis (37).

Third, the serotonergic system has also been implicated in all three RMDs. However, we do not consider this body of research as being contradictory to the simultaneous involvement of GABAergic neurosteroids. Indeed, there is considerable evidence suggesting that the serotonergic system is directly modulated by these neurosteroids, including ALLO (46, 66). In sum, clear linkages between the GABAergic and the serotonergic systems have been found in the hippocampus, where serotonin neurons frequently end at inhibitory GABAergic interneurons (67). Also *in vivo* administration of low doses of a 5HT1A receptor agonist that comes with anxiolytic effects enhances GABA stimulated ClP^- Puptake in cortico-hippocampal synaptoneurosomes (68).

Recent literature presented earlier in this manuscript and also in the following closes the circle to chronic stress or trauma in childhood and youth, that has been established as a clear risk factor for RMDs. Life stress that causes developmental insults alters exactly those GABA-stimulated chloride mechanisms in CRH neurons that constitute a key mechanism in the GABAergic control of the HPA axis. These insights link to and build forth on another research tradition that might benefit research on RMDs: research on the psychobiology and molecular genetics of resilience (187). The stress resilience of women with RMDs seems to be severely compromised, which relates to the very few existent studies on chronic stress and early life stress reported earlier in this article in this context. In a recent and comprehensive review on this topic, Hodes and Epperson (188) elaborate on the existing findings concerning the difference between men and women in response to significant life stress. The findings presented in this review confirm that life stress leading to developmental insults, in particular during childhood and puberty, is unmasked in women during hormonal fluctuations in reproductive transitions. In women, this then manifests in stress-related disorders such as depression, anxiety and posttraumatic stress disorder. In contrast, prenatal and early postnatal stress in men tends to manifest in other symptoms, such as those pertaining to the autism spectrum disorders as well-attention-deficit/ hyperactivity disorder. Further research in this particular context of reproductive transitions and life stress in women could hold valuable keys for improving the mental health of women affected by RMDs.

Also, the brain imaging evidence presented in this article shows that there is far more research needed to get a clear picture of shared activation patterns in RMDs, as the studies for each RMD are to heterogenous. In sum, a couple of aspects have to be kept in mind when drafting a common etiological model for RMDs: a clear difference in brain activation patterns has been found between adult RMDs and premenarchal mood problems, in that the latter was more akin to MDD. So it seems sensible to draft a common etiological model for adult RMDs only. In brain imaging studies GABA also played a central role, in that GABAergic fluctuations were clearly tied to fluctuations in the reproductive hormonal system. Also, decreasing ALLO levels were found to be linked to changes in brain activation pattern. So, brain imaging research also warrants the inclusion of the GABAergic system and its activating neurosteroids into an etiological model of RMDs. Not only the GABAergic mechanism at the level of the PVN, activation patterns in the hippocampus, but also compromised connectivity between the amygdala and the prefrontal cortex played a major role in RMDs. Some researchers found an identical network dysfunction and blunted brain activation patterns for all RMDs. Concerning serotonergic functioning hypogonadal states were found to come with greater serotonergic uptake and decreased serotonin transporter binding blunting serotonergic functioning in brain imaging studies. Both, the serotonergic as well as the GABAergic system need to find a place in a joint etiological model.

In light of the above, in **Figure 1** we present a graphical abstract that could serve as a stepping stone for etiological models for RMDs. In this graphic we integrate the supposed shared mediators and moderators of RMDs. The model expands on reproductive steroid hormone sensitivity as a concept suggesting that rather than absolute steroid hormone levels, it is the sensitivity toward relative changes in reproductive steroid hormones (indicated by plus and minus symbols) which drives affective, cognitive, and physiological outcomes (11). These outcomes can also be expected to be closely related and depend on situation-level variables of women such as psychosocial stressors, as well as person-level variables suches the genetic predisposition and epigenetic modifications of the systems introduced and labeled in the following.

Therefore, we wish to add a focus on a sensitivity to stress via altered $GABAR_A$ Rreceptor and ALLO functioning on a central nervous system level. Thus, we find it helpful to propose to expand on the concept of reproductive steroid hormone sensitivity toward introducing a more integrative concept of "steroid hormone sensitivity": We include both, a sensitivity in the reproductive steroid system (RSS) as well as what we label "the stress steroid system" (SSS). The dynamics between those two steroid systems needs in-depth investigation. There are to date no integrative data on these neuroendocrine dynamics and their influence on women's everyday life. Shared sensitivity to normal reproductive steroid and neurosteroid concentrations seems the focal point in the Stress steroid system for all RMDs. Therefore, we position $GABAR_A$ Rreceptor composition as the central switch between the RSS and the SSS in our graphical abstract, as this receptor regulates both systems as reviewed earlier in this manuscript. The composition and plasticity of this receptor seems key in whether the cross-talk between the two steroid systems is flexible and adaptable during hormonal fluctuations. Whether a woman with a vulnerable genetic or epigenetic set-up including compromised GABAergic modulation develops RMDs then depends on the social context, including its stressors and supportive factors.

CONCLUSION

For all three RMDs manifesting in adult women's life—PMDD, PPD and perimenopausal depression—there is strong evidence that biological factors (e.g., hormonal, genetic) and psychosocial factors (e.g., daily-life burden/stressors) interact to predict depressive symptoms. However, so far, much of the research has tended to examine these mood disorders from one perspective or the other, failing to consider how an individual's biological vulnerability may interact with her social environment to predict the risk for depression. As a result, clinicians frequently have a limited view of RMDs: obstetricians/gynecologists may tend to view these disorders as being purely hormonal, responsive only to pharmacological intervention, while counselors and social workers may fail to appreciate the degree to which biological influences play a role.

Furthermore, despite increasing evidence that the RMDs may have much in common, they are still being investigated in isolation, with research teams specializing in one disorder or the other. This is also reflected in the diagnostic manuals ICD [e.g., (111)] and DSM-5 (71), where these disorders are spread across different diagnostic categories of codes. RMDs still have to find their adequate place in diagnostic manuals and it is continued research on RMDs that is paving the way for this.

Expanding our perspective toward the more comprehensive concept of "Steroid Hormone Sensitivity" that integrates interactions between both, the reproductive steroid system (RSS) and the stress steroid system (SSS) might serve as a neuroendocrine basis for a better understanding of the underlying differential stress sensitivity experienced by affected women in daily life. Psychosocial stress effects manifesting in affective as well as cognitive symptoms may be of particular importance in these mechanisms. Compromised in their cognitive functioning and in turn stressed by these disabling symptoms, these women may spiral down in a vicious circle between affective, physiological and cognitive symptoms ultimately resulting in what we diagnose as RMDs. Researchers in both research traditions on depresssion- those on reproductive steroids as well as those on stress steroids- seem to be more and more turning toward abnormalities in receptor plasticity. The $GABAR_A$ RReceptor could be the key switch between the reproductive and the stress steroid system with mounting evidence at a subunit level within this receptor complex: a compromised plasticity required during hormonal fluctuations. Concluding from our review, the role of the δ subunit within the $\alpha R_4 R\beta\delta$ GABA Receptor seems to be key in a successful endocrine modulation of reproductive transitions in an environment marked by social stressors.

Working with a common etiological model may not only help us all to proceed in our joint understanding of RMDs, but it may also facilitate the development of new therapeutic approaches combining psychosocial and neuroendocrine aspects. Detecting psychosocial stressors such as the lack of support and finding solutions for them is one of those aspects. From a neuroendocrine point of view, elevating ALLO levels has been discussed as a therapeutic approach to stress-related disorders (46). The FDA approval of a formulation of ALLO (Brexanolone) in the treatment of postpartum depression has, therefore, broad implications for our field. Others have also focused on the translocator protein (18kDa) (TSPO) which is key for neurosteroidogenesis (189) or a novel, synthetic, neuroactive steroid SGE-516 to improve postpartal depression-like symptoms in mice (190). Overall, the role of $GABAR_A$ RReceptor composition for the activation of the receptor by neurosteroids (37, 46) should be at the center of pharmacological endeavors. In this context there is still far more in-depth evidence needed for the epigenetic regulation of GABAergic transmission. In depression research addressing GABAergic deficits means moving from the mere treatment of the symptoms of depression toward correcting causal neurochemical imbalances.

From a methodological point of view, we encourage the use of parallel methodologies across the three RMDs and suggest testing for common neuroendocrinological, genetic and psychosocial contributors to these disorders. Several methodological challenges will have to be carefully taken into

account: the adequate measurement points in all disorders; interaction effects between reproductive steroid hormones and sex hormone binding globulin (SHBG), dehydroepiandrosterone DHEA and androgen measurement; inflammation markers; the choice of biomarkers for genetic phenotyping (such as serotonin, genotyping receptors, but also transporters); other neurotransmitters, targeting GABA receptors and ALLO in humans. With respect to psychosocial factors, confounding factors such as aging and self-image in the case of menopause or anxiety in younger women having to deal with family and workload have to be considered too. The growing high-risk demographic of single working mothers would be a good example, as the prevalence of depression in this group is significantly higher than in married controls due to chronically high stress levels (191). Sub-clusters of patients within the respective RMDs will have to be carefully distinguished as may have become clear in the course of this review. Current research that is exploring the crosstalk between Glucocorticoids, Reproductive Hormones and Immunity (192) may also benefit from picking up this thread of research on RMDs.

Ultimately, our review is meant to further establish RMDs as a phase-sensitive distinct clinical entity warranting the development of a new diagnostic category on RMDs in future editions of ICD and DSM. In particular, in the face of the compelling evidence available on perimenopausal depression, it is urgently necessary to insert a respective diagnostic category, as even the upcoming ICD-11 does not make any reference to this particular RMD. Integrating our knowledge on these health issues of women in a bigger picture allows us to take a psychoneuroendocrinologically informed stance on fundamental societal questions that have long been of pressing relevance. These include the differential social support and stress profiles in the context of different depression prevalence between men and women (120) and involves factors like the increase in stress-related diseases world-wide.

As we tried to show in this review, the hormonal as well as the psychosocial environment of the menopause transition is clearly implicated in the development of for instance perimenopausal depression. There is mounting evidence that the presence of psychosocial stress may interact with this hormonal environment to confer an increased mood vulnerability. Taking such a psychoneuroendocrinologically informed stance also calls for new teaching contents in medical and psychological faculties as well as a new type of practices for Gynecological Psychoneuroendocrinology (193). In this new type of practice treatment options will be offered that integrate neuroendocrinological knowledge with psychiatric, gynecological, urological, and social evidence as well as the latest insights of sexual medicine. This new type of practice supports women in finding adequate solutions to their problems, an extraordinarily demanding integrative effort of various medical, social and psychological services that women were left alone with for most of this world's medical history. In research, teaching and practice that takes such a multidisciplinary stance, a whole array of aspects can be taken into account in finding health solutions for women. For instance, also the aspects of pain and inflammation that are linked to depression (194) has never been elucidated in its link to altered GABAergic mechanisms found in RMDs. Neuro-orthopedic, gynecological as well as urological inflammations such as cystitis often represent masked depressive processes. These are aspects which would greatly benefit progress on RMDs in research and practice if addressed in such a multidisciplinary way. On the basis of a longitudinal, multi-disciplinary perspective on RMDs that takes psychosocial and neuroendocrine factors into account, far more effective prevention and intervention strategies including pharmacological, psychotherapeutic, and psychosocial approaches may be developed, with the aim to improve mental health of the many women in this world affected by reproductive mood disorders.

AUTHOR CONTRIBUTIONS

SS-S and BD: conception and design of the work. SS-S, JG, TE-M, SM-B, KS, RS, A-LZ, UE, and BD: drafting the article. SS-S, BD, and UE: critical revision of the article. SS-S, JG, TE-M, SM-B, KS, RS, A-LZ, UE, and BD: final approval of the version to be published. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to thank the reviewers for their immensely helpful input. In addition, on a personal note, SS-S wishes to thank her beloved Schweizer-Schubert Family for their love and support during the making of this manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 12 June 2019
doi: 10.3389/fpsyg.2019.01362

Pubertal Maturation and Trajectories of Depression During Early Adolescence

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OPEN ACCESS

Edited by:
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Reviewed by:
Laura M. Dimler, Regent University, United States
Sarah Whittle, The University of Melbourne, Australia

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Specialty section:
This article was submitted to Psychology for Clinical Settings, a section of the journal Frontiers in Psychology

Received: *15 October 2018*
Accepted: *24 May 2019*
Published: *12 June 2019*

Citation:
McGuire TC, McCormick KC, Koch MK and Mendle J (2019) Pubertal Maturation and Trajectories of Depression During Early Adolescence. Front. Psychol. 10:1362. doi: 10.3389/fpsyg.2019.01362

Beginning at puberty, prevalence of depression in females rises dramatically. The physical changes of puberty coincide with a period of social flux, during which relationships become less stable and more prone to conflict. While this social upheaval is normatively distressing for girls, it may be especially so for girls with cognitive styles that leave them more susceptible to depression. The present study investigated depressive symptoms at two time points during early pubertal maturation. N = 110 girls (M_{age} = 11.57, SD = 0.98) reported on depressive symptomology, pubertal maturation, ruminative coping style, frequency of peer conflict, and rejection sensitivity. Multivariate analyses suggest more advanced pubertal development and greater rejection sensitivity at Time 1 predicted higher levels of depressive symptoms at Time 2, after accounting for baseline levels of depressive symptoms and all other social and cognitive correlates of depression. This effect was also found in early maturing girls. Menarche status was not significant. Since menarche occurs toward the end of puberty, results suggest that risk for worsening depression is not associated with completing puberty, or with menstruation itself. Rather, increases in depressive symptoms seem to be associated with physical changes that emerge early in the pubertal transition, especially for early maturing girls, paired with anticipatory concerns about social rejection.

Keywords: adolescent, female, menarche, puberty, depression, rejection, peers

INTRODUCTION

The major reproductive milestones in women's lives – puberty, menstruation, childbirth, and menopause – all encompass major biological, psychological, and socioemotional changes. Puberty, the first reproductive milestone, refers to the physical growth and sexual maturation that indicates passage from childhood into adolescence. Because puberty occurs earlier in the life span, the complex changes and experiences during this period have the potential to alter developmental trajectories. Changes at puberty are especially notable because of the concurrent

shift in depression vulnerability. Findings from epidemiological research suggest that rates of depression are approximately equal between sexes before the onset of puberty, after which, rates for depression in girls are approximately twice that of boys (Bromet et al., 2011; Salk et al., 2017). While puberty alone may play an integral role in the increased risk of depression, the psychological changes associated with adolescence are also intimately intertwined with contemporaneous biological and sexual maturation. The present study investigates how biopsychosocial changes during puberty contribute to and predict exacerbations of depressive symptoms in girls during the early adolescent window.

There are a variety of changes girls must adjust to during puberty. The onset of puberty is marked by significant rises in adrenal and gonadal hormones and the development of primary and secondary sex characteristics. It takes, on average, 4–5 years to progress through puberty (Finer and Philbin, 2013). *Pubertal status* is conceptualized as the level of development through visible bodily shifts in breasts, body shape, height, skin, and body hair. More advanced pubertal status, or physical development, has been linked to depressive disorders and is thought to help explain gender differences in rates of depression (Hankin and Abramson, 2001; Conley and Rudolph, 2009; Lewis et al., 2018). *Pubertal timing*, or the relative status of one's development compared to same-sex peers, has also been shown to have significant associations with the development of internalizing symptoms (Ullsperger and Nikolas, 2017). Prior research suggests that early maturing girls tend to exhibit more severe levels of depressive symptoms than girls who mature at the same time as or later than most peers (Negriff and Susman, 2011; Graber, 2013; Lewis et al., 2018; Mendle et al., 2018; Hamlat et al., 2019).

In addition to tracking the development and timing of visible indicators of pubertal maturation (e.g., skin changes, hair growth, etc.), it may be important to consider menarche. Menarche, or the start of menstruation, is one of the final pubertal milestones and represents the completion or near-completion of sexual maturation. Some research suggests age at menarche shows different patterns of association with symptoms of depression and other psychopathologies compared to other pubertal indicators like breast development (Harden et al., 2012; Carter et al., 2013; Kretsch et al., 2016). These findings demonstrate the need to question whether entering, enduring, or completing puberty poses a greater risk for depression.

The onset of puberty typically marks the entry into adolescence, or the developmental period that connects childhood and adulthood. Early adolescence, or the period from the onset of puberty to 14 or 15 years of age (Sawyer et al., 2012), represents a distinctly turbulent period of socioemotional change. The shifting social landscape during this time places greater importance on peer relationships and social status. With this increased focus on social relationships, girls are also susceptible to experiencing more negative social stressors that may contribute to vulnerability for depression. As girls renegotiate friendships and relationships, interpersonal conflicts and fears of social rejection can become more prominent. Conflict with peers contributes to internalizing symptoms in adolescent girls (Purdie and Downey, 2000). In addition, rejection sensitivity, or the tendency to expect and readily perceive rejection from others, has been associated with increased psychopathology in adolescence and adulthood (Purdie and Downey, 2000; Ayduk et al., 2001; Marston et al., 2010; Zhou et al., in press). Individuals who experience higher levels of rejection sensitivity are more likely to perceive rejection in the face of ambiguous stimuli and are more sensitive to both the potential for and likelihood of being rejected by others. Further, girls with high rejection sensitivity are particularly likely to experience peer conflict and relationship difficulties (Purdie and Downey, 2000). Research suggests that being liked by peers predicts a reduction of rejection sensitivity in adolescents (London et al., 2007). Consequently, higher levels of rejection sensitivity and peer conflict during puberty could color perceptions of peer relationships as particularly fraught and uncertain, which in turn could generate more interpersonal stress and vulnerability for depression.

These myriad biological and socioemotional changes may be particularly distressing to girls utilizing maladaptive coping strategies. Rumination is an emotion regulation strategy that may be used to cope with stressors by perseverating on the causes, consequences, and factors involving one's distress. Because rumination is linked to negative affect, it has been studied as a precursor to depression in childhood, adolescence, and adulthood (Nolen-Hoeksema et al., 1993; Gibb et al., 2012; Gomez-Baya et al., 2017), and has been implicated in the rise in depressive symptoms for girls at puberty (Mezulis et al., 2014; Alloy et al., 2016). In addition, there is evidence to suggest that adolescent girls are more likely to ruminate than preadolescent girls (Hampel and Petermann, 2005) and that early adolescents who ruminate tend to report more difficulties in their peer relationships and greater symptoms of internalizing (McLaughlin and Nolen-Hoeksema, 2012). Given the socially tumultuous nature of adolescence, girls who experience more peer conflict and are higher in rejection sensitivity may be made further vulnerable by a cognitive style that has been found to increase negative affect (Nolen-Hoeksema et al., 1993).

The Present Study

The present study investigated trajectories of depressive symptoms specifically during early reproductive maturation. Because puberty is a time when interpersonal interactions become increasingly salient and fraught for girls, we sought to examine the contributions of common biopsychosocial correlates of depression – rumination, rejection sensitivity, peer conflict, and level of pubertal development – with exacerbations in depressive symptomology over a 4-month period. We also examined these biopsychosocial correlates with respect to pubertal timing, considering whether these symptom trajectories might be intensified in girls who matured earlier than peers. While previous research has identified the interplay of pubertal and psychosocial changes in relation to psychopathology (see review articles; Musliner et al., 2016; Shore et al., 2018), very few studies, to date, have explored the constellation of variables in the present study and clarified mechanisms of risk and vulnerability during the early adolescent transition. We therefore targeted

two specific research questions. First, are girls with more advanced pubertal status more likely to experience depressive symptoms at follow-up, after accounting for baseline levels of depression, rumination, rejection sensitivity, and interpersonal difficulties? Second, does this pattern of findings hold for early maturing girls?

MATERIALS AND METHODS

Participants

Participants comprised $N = 110$ girls ($M_{age} = 11.57$, range = 9–14) recruited from a research partnership established with (New York State 4-H) youth summer programs between 2015 and 2017. All girls in the target age range were invited to participate Girls were administered a questionnaire battery at the time of recruitment and a 4-month follow-up survey. For the purposes of this study, only participants who completed the follow-up survey were included in analyses. In this sample, youth self-identified as primarily European American (80%), Black or African American (2.7%), American Indian or Native American (3.6%), East Asian/Pacific Islander (3.6%), Southeast Asian (0.9%), or another racial/ethnic background (7.3%). Two percent of girls self-identified as Hispanic/Latina. The study was approved by the Institutional Review Board at Cornell University, IRB Protocol #1207003173. Parental/guardian written informed consent and youth written assent were obtained from participants.

Measures

Reproductive Maturation

The Pubertal Development Scale (PDS; Petersen et al., 1988) was used to assess pubertal status, pubertal timing, and menarcheal status. Pubertal status was operationalized using four items assessing changes in height, body hair, skin (i.e., acne), and breast growth. Items were measured using a four-point Likert scale from 1 (= *has not yet begun*) to 4 (= *seems completed*) and summed for a composite score. The mean composite PDS score in this sample was 9.90 (SD = 2.65, range = 3–16), with higher scores indicating greater overall physical development. To operationalize pubertal timing, composite PDS scores were standardized according to year of chronological age, so that higher scores indicate greater levels of physical development relative to girls of the same age. Menarcheal status was assessed using one dichotomous indicator from the PDS: "Have you begun to menstruate (get your period)?"

Depression

The Center for Epidemiological Studies-Depression Scale for Children (CES-DC; Radloff, 1977) is a 20-item self-report questionnaire used to assess depressive symptomatology in children. Scores on the CES-DC range from 0 to 60, with higher scores indicating greater endorsement of depressive symptoms. A score of 16 is typically used to signify a clinically relevant level of symptoms. In this sample, the mean score at baseline was 15.72 (SD = 11.07, range = 0–56). The mean score at 3-month follow-up was 14.74 (SD = 11.53). Internal reliability was good at Time 1 ($\alpha = 0.90$) and excellent at Time 2 ($\alpha = 0.93$).

Peer Relations

The Index of Peer Relations Scale (IPR; Hudson, 1992; Forte and Green, 1994) was used to ascertain the severity of peer relationship problems and social adjustment difficulties. The IPR comprises 25 statements rated on a seven-point Likert scale, ranging from 1 (none of the time) to 7 (all of the time). The present study modified the wording of the IPR to substitute the phrase "kids my age" for "peers." Sample items include: "Kids my age don't seem to even notice me," "I really feel left out of the group of kids my age," and "I hate kids my age." Scores on the IPR range from 0.67 to 100, with higher scores indicative of greater peer conflict. A score at or above 30 typically signifies a clinically relevant level of peer problems and a score at or above 70 indicates severe distress. In the current study, the mean score was 28.39 (SD = 16.46, range = 0–84.67). Internal reliability was excellent ($\alpha = 0.95$).

Rejection Sensitivity

The Children's Rejection Sensitivity Questionnaire 6-Item Form (CRSQ; Downey et al., 1998) was used to measure children's propensity to respond defensively to social rejection or anticipated social rejection. Children are asked to determine the degree of anxiety and anger they would feel in six social rejection scenarios, with responses scored on a six-point Likert scale, from 1 (not nervous/mad) to 6 (very, very nervous/mad). The CRSQ also queried perceived likelihood of a positive outcome for each scenario, with responses scored on a six-point Likert scale, from 1 (YES!!!) to 6 (NO!!!). Anger and anxiety items were multiplied separately by each likelihood item, and all six item scores were averaged to create a total score. Higher scores are indicative of greater rejection sensitivity. The total rejection sensitivity mean score in the sample was 17.36 (SD = 8.97, range = 3–49). The anger sub-scale mean was 7.58 (SD = 4.39; range = 1.17–25.67) and the anxiety sub-scale mean was 9.78 (SD = 5.14; range = 1.83–25). Internal reliability was good ($\alpha_{total} = 0.85$; $\alpha_{anger} = 0.79$; $\alpha_{anxiety} = 0.80$).

Ruminative Coping

Ruminative coping was assessed using the Ruminative Response Scale of the Children's Response Styles Questionnaire (CRSQ-R; Abela et al., 2002). Sample items include: "When you're sad, you think about how sad you feel" and "When you're sad, you think about a recent situation wishing it had gone better." Scores on the CRSQ-R ranged from 0 to 39, with greater scores indicating higher ruminative thinking. The mean score was 13.58 (SD = 9.01, range = 1–39). Internal reliability was good ($\alpha = 0.89$).

Analytic Plan

We investigated how biopsychosocial factors predicted depression at Time 2 using two three-stage hierarchical linear

regressions. The pubertal status model predictor variables included rejection sensitivity, rumination, peer conflict, pubertal status (with age, scaled in months and years, as a covariate). The pubertal timing model included the same predictor variables; however, pubertal timing was included instead of pubertal status and age was not included as a covariate, as chronological age is taken into account in the operationalization of pubertal timing. Descriptive analyses and hierarchical linear regressions were conducted using SPSS 25 (IBM Corp, 2016). Continuous predictor variables were centered at their means prior to analysis. All variables met assumptions of linear regressions. Step 1 was a simple linear regression predicting depressive symptoms at Time 2 from depressive symptoms at Time 1. Depressive symptoms at Time 1 were entered at Step 1 to control for depressive symptoms at Time 2. At Step 2, cognitive and social risks for depression (peer conflict, rejection sensitivity, and rumination) were added. At Step 3, reproductive maturation variables (i.e., PDS scores and menarche status) were added to the model. The variables were structured to assess whether the pubertal variables predicted depression above and beyond the effect of the social and cognitive risk factors and baseline levels of depressive symptoms.

RESULTS

Descriptive Analyses

Table 1 depicts descriptive statistics and bivariate Pearson correlations of all variables. The psychosocial variables (rumination, rejection sensitivity, and peer conflict) were significantly intercorrelated. Reproductive maturation variables were not significantly correlated with the psychosocial variables, except for a small correlation between pubertal status and rumination (r = 0.20). Time 2 depressive symptomatology was significantly correlated with all biopsychosocial predictor variables, ranging from menstruation and pubertal timing (r = 0.23) to Time 1 depressive symptomology (r = 0.58).

Hierarchical Regressions

Full results of the hierarchical regression models are summarized in **Tables 2** and **3**. Girls reporting higher levels of depressive symptomology at Time 1 were more likely to report experiencing more depressive symptomology at follow-up (β_{status} = 0.36, p = 0.001; β_{timing} = 0.36, p = 0.001). Additionally, higher levels of rejection sensitivity at Time 1 significantly predicted higher levels of depressive symptoms at Time 2 (β_{status} = 0.29; p = 0.005; β_{timing} = 0.27; p = 0.008), even after accounting for Time 1 symptoms. Neither rumination (β_{status} = 0.02; p = 0.83; β_{timing} = 0.04; p = 0.73) nor peer conflict (β_{status} = 0.00; p = 0.99; β_{timing} = 0.04; p = 0.65) were significant predictors of depression in either the pubertal status or pubertal timing models. Girls with a more advanced pubertal status at Time 1 were more likely to report higher levels of depressive symptoms at Time 2 (β_{status} = 0.17; p = 0.04), after accounting for baseline levels of depressive symptoms and social and cognitive correlates of depression. Girls with early pubertal timing at Time 1 were also more likely to report higher levels of depressive symptoms at Time 2 (β_{timing} = 0.17; p = 0.03), after accounting for baseline levels of depressive symptoms and social and cognitive correlates of depression. Menarche status was not a significant predictor of depressive symptoms at Time 2 in either model (β_{status} = 0.03, p = 0.72; β_{timing} = 0.14, p = 0.07).

Supplementary Analyses

Although our sample was relatively limited in racial and ethnic diversity (see discussion, below), a supplementary set of models for both pubertal status and pubertal timing were run with race/ethnicity included as a covariate. Race/ethnicity was coded in two ways: (1) all race/ethnicity options as separate dummy variables and (2) white and nonwhite dummy variables. Results did not change.

DISCUSSION

Depression in adolescent girls is both endemic and worrisome. The extant literature has examined psychosocial correlates and their associations with the trajectory of depressive symptoms (Mezulis et al., 2014; Musliner et al., 2016; Ellis et al., 2017; Shore et al., 2018). The present study extends prior findings by investigating how multiple indicators of pubertal maturation and psychosocial risk factors contribute to and predict exacerbations of depressive symptoms in early adolescent girls. Our results suggest an important coupling of pubertal maturation and rejection sensitivity. Girls with a more advanced pubertal

TABLE 1 | Descriptive statistics.

	1	2	3	4	5	6	7	8
T1 depression	–							
Rumination	0.69***	–						
Peer conflict	0.46**	0.34**	–					
Rejection sensitivity	0.52**	0.46**	0.62**	–				
Pubertal Development Scale	0.16	0.20*	0.13	0.07	–			
Age	0.12	0.12	0.17	0.12	0.39**	–		
Menstruation	0.11	0.07	0.04	0.05	0.41**	0.44**	–	
T2 Depression	0.58***	0.47**	0.42**	0.51**	0.34**	0.34**	0.23*	–
Mean	15.73	13.58	28.40	17.36	9.90	11.57	1.87	14.74
SD	11.07	9.01	16.46	8.97	2.65	0.98	1.37	11.53

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 2 | Pubertal status regression estimates predicting Time 2 depressive symptomology.

Step	Variable	β	SE	R^2	$\Delta R2$
Step 1	Depressive symptoms	0.36**	0.11	0.34	
Step 2	Peer conflict	0.00	0.07	0.41	0.07***
	Rumination	0.02	0.13		
	Rejection sensitivity	0.29**	0.13		
Step 3	Pubertal development	0.17**	0.36	0.50	0.09***
	Age	0.19**	0.98		
	Menarcheal status	0.03	2.12		

*β, standardized coefficient; SE, standard error; R^2, percentage of variance accounted for at each step; ΔR^2, change in R^2 at each step. *p < 0.05; **p < 0.01; ***p < 0.001.*

TABLE 3 | Pubertal timing regression estimates predicting Time 2 depressive symptomology.

Step	Variable	β	SE	R^2	ΔR^2
Step 1	Depressive symptoms	0.36**	0.11	0.34	
Step 2	Peer conflict	0.04	0.07	0.40	0.07*
	Rumination	0.04	0.13		
	Rejection sensitivity	0.27**	0.13		
Step 3	Pubertal timing	0.17*	0.93	0.46	0.06**
	Menstrual status	0.14	1.95		

*β, standardized coefficient; SE, standard error; R^2, percentage of variance accounted for at each step; ΔR^2, change in R^2 at each step.*p < 0.05; **p < 0.01; ***p < 0.001.*

status, earlier pubertal timing, and those who express a high level of concern about social rejection were more likely to report worsening depressive symptoms. Menarcheal status, peer conflict, and ruminative coping were not significant predictors of later depressive symptoms.

A strength of the present study is that it provides a foundational basis that begins to disentangle the complex role of puberty in concert with common psychosocial predictors of psychopathology. Our sample offered the opportunity to examine three indicators of pubertal development: pubertal timing, pubertal status, and menarcheal status. Consistent with our hypotheses, both pubertal status and pubertal timing played an independent role in depressive symptoms over and beyond the effect of baseline levels of depressive symptoms and psychosocial predictors: girls who were further along in the pubertal process and girls who were further along relative to girls of the same age were at a greater risk of reporting depressive symptomology. Completing menarche did not confer additional risk. Our results are a snapshot into early adolescence and underscore the need for further work to examine pubertal processes within a larger, longitudinal sample. Future studies can build upon the present study and identify how specific mechanisms of puberty uniquely contribute to exacerbated risk in girls.

Understanding why reproductive maturation poses psychological risk is a complicated task for researchers. Although hormonal changes are not singularly associated with increases in depression (Soares and Zitek, 2008), studying reproductive maturation can elucidate the relationship between hormones, heightened sensitivity to hormone changes, and vulnerabilities for the onset or worsening of depression. Prior research has shown that hormone changes throughout the menstrual cycle are associated with increases in stress reactivity, negative cognitive appraisals, and internalizing symptoms (Albert et al., 2015; Kiesner et al., 2016; Mulligan et al., 2018). Our study confirmed that even very early reproductive changes during puberty predict decrements in mood. Notably, menarche status did not predict depressive symptoms, which is not inconsistent with existing literature. Menarche, although a useful marker for development, does not indicate hormone levels or cyclical reproductive hormone change, and is only one indicator of pubertal development. The menstrual cycle can take between 3 and 4 years to stabilize post-menarche (Widholm and Kantero, 1971; De Sanctis et al., 2019). Therefore, the onset of menstruation itself is not a good indicator of an individual's hormonal levels or the stability of their menstrual cycle and cyclical hormone change, which may explain why menarche status did not map onto depressive symptoms beyond pubertal status. Future research could explore how both the increase and variability of hormone levels during this period are associated with cognitive vulnerabilities to depression. Exploring the specific psychosocial changes that pubertal status signals can also inform our understanding of risk for depression exacerbation during the early pubertal transition. Additionally, studying the effects of hormonal changes occurring during adrenarche may further clarify the role that hormones have on depressive symptoms. A meta-analysis of adrenarche and mental health found an association between adrenarche and both internalizing and externalizing symptoms (Byrne et al., 2017); however, the authors also noted the deficit of research studying this period of early adolescent development.

Consistent with prior literature (Tops et al., 2008; Liu et al., 2014; Zhou et al., in press), our results also highlight the importance of adolescents' anticipatory concerns about how others will perceive and respond to them in social settings. Recent work has examined the mechanism by which rejection sensitivity is related to depression, suggesting that stress generation may mediate the relationship between depression and rejection sensitivity (Liu et al., 2014). Specifically, individuals high in rejection sensitivity may behave in ways that elicit more conflict with peers, leading to greater interpersonal stress. Puberty represents a time of myriad new stressors, which could help explain how rejection sensitivity influences depression through increased relationship stress and sensitivity to rejection in these precarious peer relationships. Future work could examine how specific types of relationships could serve to ameliorate or worsen rejection sensitivity, stress, and depression.

Perhaps most notably, we did not find that either rumination or peer conflict were necessarily associated with worsening depression over time, after accounting for baseline levels of depressive symptoms. This result is surprising, given prior research that suggests rumination exacerbates depressive

symptoms (Mezulis et al., 2014; Grierson et al., 2016) and mediates the effect of pubertal timing on depression (Alloy et al., 2016). One possibility is that rumination is not as robust a predictor of subsequent depression symptoms when more biopsychosocial indicators are simultaneously included in analysis. It is also possible that many of these variables are closely related and more complex modeling techniques, such as structural equation modeling, may be needed in order to tease apart relationships with depression. Finally, rejection sensitivity may serve as either a moderator or mediator between rumination and depression (Hilt et al., 2017). Findings from the adult literature suggest that rejection sensitivity is prospectively associated with rumination (Pearson et al., 2011), which suggests that individuals prone to perceiving rejection may be more likely to dwell on that rejection. Further research with additional time points may better clarify the relationship between these social-cognitive processes throughout adolescence.

Our study offers new insights into the early adolescent period, but – like all research – it is not without its limitations. These include the comparatively small sample size and short longitudinal follow-up time. In addition, while our sample demographics are consistent with the demographics of the region in which the data were collected, the sample is also limited by a lack of racial/ethnic diversity and cannot contribute to a broader understanding of how becoming reproductively mature may differ meaningfully for girls depending on their background. Research on pubertal development can be furthered by a focus on racially and ethnically underrepresented groups (Mendle et al., 2019), which have been historically understudied. Additionally, future studies should seek to better understand the experience of sexual minorities and how sexual and gender identity may be related to mental health during the pubertal transition. Finally, our analyses focus on girls, but it could be argued that a comparison across sexes might provide the best understanding of the unique vulnerabilities associated with female reproductive maturation.

Given these limitations, we view our study as a preliminary investigation into this topic, and hope that it serves as a foundation for future research. Analyzing depressive symptoms, rejection sensitivity, rumination, and peer conflict in tandem with reproductive maturation across two time points allows for a clearer picture of the potential causes and consequences of psychosocial factors commonly found to underlie depression. A follow-up investigation of these factors is an important future direction of this work in disentangling how puberty is a period of increased risk for worsening depressive symptoms in girls.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Cornell University Institutional Review Board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Cornell University Institutional Review Board.

AUTHOR CONTRIBUTIONS

JM conceived and designed the experiment. TM, KM, and MK contributed to data collection. TM analyzed the data. TM and KM wrote the paper. JM, TM, KM, and MK provided feedback and revision of the text.

FUNDING

Data collection for this study was funded by the Program for Research on Youth Development and Engagement (PRYDE) at Cornell University, supported by a gift from Rebecca Q. Morgan.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 29 November 2018
doi: 10.3389/fpsyt.2018.00547

Early Life Abuse Moderates the Effects of Intranasal Oxytocin on Symptoms of Premenstrual Dysphoric Disorder: Preliminary Evidence From a Placebo-Controlled Trial

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OPEN ACCESS

Edited by:
Roberto Viviani, Universität Innsbruck, Austria

Reviewed by:
Rodrigo Machado-Vieira, National Institute of Mental Health (NIMH), United States
Erika Comasco, Uppsala University, Sweden

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Specialty section:
This article was submitted to Psychopathology, a section of the journal Frontiers in Psychiatry

Received: *18 April 2018*
Accepted: *12 October 2018*
Published: *29 November 2018*

Citation:
Walsh EC, Eisenlohr-Moul TA, Pedersen CA, Rubinow DR, Girdler SS and Dichter GS (2018) Early Life Abuse Moderates the Effects of Intranasal Oxytocin on Symptoms of Premenstrual Dysphoric Disorder: Preliminary Evidence From a Placebo-Controlled Trial. Front. Psychiatry 9:547. doi: 10.3389/fpsyt.2018.00547

Background: Although intranasal oxytocin (OXT) has been proposed to be a promising treatment for some psychiatric disorders, little research has addressed individual difference factors that may predict response to OXT. One such factor is early life abuse (ELA), which has widespread influences on social-emotional processing and behavior. This single-blind, placebo-controlled crossover trial examined the role of ELA in shaping the effects of intranasal OXT (vs. placebo) on daily behavioral symptoms in women with three or more prospectively-diagnosed cycling symptoms of premenstrual dysphoric disorder (PMDD).

Methods: Participants were ten women with PMDD ($n = 8$) or subthreshold PMDD ($n = 2$), who had experienced ELA prior to age 13 ($n = 5$) or no ELA ($n = 5$). They completed two study visits during the late luteal (premenstrual) phase: once following administration of intranasal OXT and once following intranasal placebo (counterbalanced). Participants then self-administered OXT or placebo at home three times per day for 5 days or until menstrual onset, and prospectively rated daily emotional symptoms of PMDD. Power was adequate to detect medium main and interactive effects.

Results: Among women with ELA, intranasal OXT (vs. placebo) increased the premenstrual emotional symptoms of PMDD, whereas among women without ELA, OXT decreased symptoms.

Conclusion: This study adds to a growing literature highlighting the importance of considering historical social contexts and traits (such as ELA) as moderators of therapeutic response to OXT.

Keywords: oxytocin, early life abuse, PMDD, emotional symptoms, interpersonal symptoms

INTRODUCTION

Premenstrual dysphoric disorder (PMDD) affects 3–8% of reproductive age women (1) and is characterized by the cyclic recurrence of affective, somatic, and interpersonal symptoms during the luteal phase of the menstrual cycle, with full remission of symptoms during the follicular phase (1, 2). Diagnosis with PMDD requires clinically significant changes in one of four core emotional symptoms: anger/irritability, depression, anxiety, and mood swings (1); further, to meet strict diagnostic criteria for PMDD as recently codified in the DSM-5, five or more unique symptoms must demonstrate this cyclical change (3). However, this requirement of five-or-more unique cycling symptoms has been criticized as being too strict, since many women experience fewer cycling symptoms that are nonetheless severe enough to cause cyclical impairment (4, 5). Degree of impairment in women with PMDD or subthreshold PMDD has been found to be equivalent to other mood disorders (1, 5). Interpersonal symptoms, such as anger/irritability and rejection sensitivity, are among the most commonly-observed symptoms in prospectively-diagnosed PMDD (6–8). Treatment with selective serotonin reuptake inhibitors (SSRIs) resolves symptoms in many women with PMDD; however, nearly 40% do not respond (9) signaling the need to develop new treatments and to better match patients to existing treatments. In the present single-blind, randomized, crossover controlled trial of intranasal oxytocin for PMDD, we examined whether early life abuse (ELA) serves as an indicator of premenstrual symptom response to OXT (vs. placebo).

Decades of research point to a role for the neuropeptide oxytocin (OXT) in the regulation of social and emotional behavior. OXT facilitates social bonding and attachment, attenuates stress responses, and reduces anxiety-like behaviors via effects on neural circuitry central to emotional and social processing (10). OXT is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus, and OXT neurons project to brain regions involved in stress regulation (e.g., prefrontal cortex), emotion and salience (e.g., amygdala), and reward (e.g., ventral striatum) (11, 12). Human neuroimaging investigations suggest OXT may improve emotion regulation and interpersonal cooperation via top-down inhibition of arousal and fear responses, and/or enhancement of reward salience, within these neural circuits (12, 13). Accordingly, intranasal OXT has been examined as a treatment in clinical samples with affective and social-cognitive deficits, with several studies reporting superiority to placebo (10, 13).

Although some studies demonstrate a benefit of intranasal OXT on social-emotional behavior and associated brain circuitry, other studies suggest that contextual factors, including history of early life abuse (ELA), heavily moderate these findings (14, 15). Following intranasal OXT administration, participants with a history of ELA show absent or diminished socioemotional benefits (16–19). Furthermore, after OXT administration, null or worsening of effects on social cognition and behavior are observed in individuals with insecure attachment styles (20, 21), which are often associated with ELA (22). These reports are corroborated by neuroimaging investigations documenting differential patterns of OXT-related brain activation or connectivity in individuals with vs. without a history of ELA (23–25). The circuits reported in these cross-sectional studies often overlap with those known to be affected by early adverse experiences and linked to disturbances in social-emotional processing (11). Consequently, ELA-related alterations in social-emotional brain circuits are hypothesized to interact with OXT to enhance the salience of negative social cues, thereby increasing behavioral proclivities for threat, vigilance, and interpersonal conflict (14). Thus, although intranasal OXT may be a promising treatment for some psychiatric disorders—and may be a rational treatment for PMDD—a personalized approach may be necessary since OXT efficacy appears to be dependent on the presence of contextual factors such as ELA.

Notably, PMDD is associated with high rates of ELA relative to controls (26) and we have previously shown that ELA predicts a clinically and pathophysiologically distinct subgroup of women with PMDD characterized by disturbances in stress-related/neuroendocrine systems (27–31). Given the high prevalence of ELA and the centrality of emotional and interpersonal impairment in PMDD (6), PMDD represents an ideal model to investigate the interactive effects of OXT and ELA on social-emotional symptoms.

Current Study

In a single-blind, placebo-controlled crossover trial in women with PMDD, half of whom had ELA, we investigated the interactive effects of intranasal OXT and ELA on daily ratings of core emotional PMDD symptoms during the symptomatic premenstrual phase. Given previous evidence that ELA diminishes the protective effects of OXT on social and emotional symptoms, we tested the hypothesis that intranasal OXT (compared to placebo) would only improve symptoms during the symptomatic premenstrual phase in women with PMDD (or subthreshold PMDD) without a history of ELA. Consistent with prior reports, we did not expect to observe premenstrual symptom improvement in women with PMDD symptoms with a history of ELA. We suspect that ELA influences behavioral response to intranasal OXT in all women, and we utilize PMDD as an ideal sample to investigate the interactive effects of ELA and OXT on affective and interpersonal symptoms. Hence, we did not include a control group in the present study.

METHODS

Participants

Recruitment took place in the triangle region of North Carolina. After participating in a PMDD diagnostic feeder study, participants were recruited via e-mail and telephone for a study on the role of oxytocin in PMDD symptoms.

Diagnoses of PMDD (or subthreshold PMDD) were made using a standardized protocol, the Carolina Premenstrual Assessment Scoring System [C-PASS; (2)], to confirm the required cyclical symptom pattern (described in detail below) in daily symptom ratings across two to four cycles. Although the C-PASS scoring system can make the strict DSM-5 diagnosis of PMDD (which requires that 5 or more symptoms, including one

TABLE 1 | Sample descriptive information by abuse history.

Variable	Full sample (*N* = 10)	PMDD + ELA (*N* = 5)	PMDD + No ELA (*N* = 5)
Age	36.6 (28–45)	35.4 (30–43)	37.8 (28–45)
Race			
White	7 (70%)	4 (80%)	3 (60%)
Black	2 (20%)	1 (20%)	1 (20%)
Central American	1 (10%)	0 (0%)	1 (20%)
Current Psychiatric Medication	4 (40%)	2 (40%)	2 (40%)
Past DSM-IV-TR diagnosis	7 (70%)	4 (80%)	3 (60%)
MDD, recurrent	1 (10%)	0 (0%)	1 (20%)
MDD, Single			
Postpartum onset	2 (20%)	1 (20%)	1 (20%)
No postpartum onset	2 (20%)	1 (20%)	1 (20%)
Alcohol abuse	2 (20%)	2 (40%)	0 (0%)
Mean premenstrual week DRSP total symptom score at baseline	72.28 (26.5)	76.97 (29.5)	67.6 (23.5)

ELA, Early Life Abuse; MDD, Major Depressive Disorder.

emotional symptom, show the required pattern of change across at least two cycles), the C-PASS can also indicate a subthreshold diagnosis for each cycle. This subthreshold diagnosis of PMDD differs from PMDD only in that the premenstrual symptom pattern does not need to occur for 5 or more symptoms; just one core emotional symptom following the pattern is sufficient for the subthreshold PMDD diagnosis. These subthreshold PMDD criteria are equivalent to what has been historically used to make the diagnosis of premenstrual syndrome and PMDD (e.g., (32)) prior to the adoption of the strict PMDD criteria in the DSM-5. Finally, since there is no evidence that women given the research diagnosis of subthreshold PMDD differ from women with PMDD with respect to severity, clinical course, or treatment response, this more inclusive threshold was used in the present study. For details on the precise protocol and thresholds used by the C-PASS, we refer the interested reader to the validation paper (2).

The final sample consisted of 10 women (ages: 28–45, $M = 36.6$, $SD = 5.6$) with a prospectively-confirmed diagnosis of PMDD ($n = 8$) or subthreshold PMDD ($n = 2$) and no other serious medical conditions. Full demographics are presented in **Table 1**. Four of five women in each ELA group met criteria for PMDD, while one in each group met criteria for subthreshold PMDD. The two women diagnosed with subthreshold PMDD each showed the required pattern across 2 months of daily ratings for three symptoms (rather than the five cycling symptoms required for PMDD). Women with ELA reported significantly more severe average premenstrual week symptoms at baseline relative to women without ELA (see **Table 1**); of note, this mean difference is controlled via the main effect of ELA in the analyses described below. Prior to enrollment, participants underwent medical and psychiatric interviews. Other current psychiatric diagnoses, as measured by the Structured Clinical Interview for DSM-IV-TR disorders (33), were exclusionary. Seven out of 10 participants met criteria for history of a past psychiatric disorder [Major Depressive Disorder, Recurrent Episode: 1; Major Depressive Disorder, Single Episode: 4 (2 with Postpartum Onset); Alcohol Abuse: 2]. Four out of 10 participants were currently prescribed daily medications for PMDD-related symptoms (non-ELA Group: sertraline x 2, ELA Group: venlafaxine, lisdexamfetamine). With regard to psychiatric diagnoses and medications, no differences were observed across ELA groups.

History of ELA was determined by a validated structured interview (34) commonly used in our previous research and defined as physical or sexual abuse prior to the age of 13. Although some of our prior work has focused on different subtypes of abuse across different age ranges (e.g., sexual abuse before the age of 16), in the present study we did not have hypotheses about specific types of abuse, and therefore used this broad definition of ELA. The of age 13 was used as a cutoff for ELA based on evidence that earlier onset of abuse is linked to greater negative outcomes in adulthood [e.g., (35)]. Physical abuse was coded as present if the participant reported ever experiencing either (1) life threat (i.e., physically attacked with the intent to kill or seriously injure), or (2) other physical abuse (i.e., beaten up, hit, burned). Sexual abuse was coded as present if the participant reported ever experiencing the following forced sexual experiences: (1) a perpetrator touching the participant's breasts, pubic area, vagina, or anus with hands, mouth, or objects; (2) making the participant touch the perpetrator's pubic area or anus with hands, mouth or objects; or (3) vaginal or anal intercourse. Fifty percent ($N = 5$) of our sample reported ELA.

All procedures were approved by the local IRB and participants provided written informed consent. Data collection ended after an unanticipated upgrade at our facility; nevertheless, due to the large number of daily observations in this design, 80% power was achieved to observe conventionally medium sized effects of both OXT and the interaction between OXT and ELA.

Study Procedures (Premenstrual Phase)

In a single-blind, placebo-controlled crossover design counterbalanced by ELA status, participants completed two laboratory visits, including functional magnetic resonance imaging scans (fMRI not reported in the present study). Each visit occurred during the late luteal (premenstrual) phase, 7–11 days following a positive urine ovulation test. Participants were asked to abstain from nicotine and alcohol for minimally 12 h, and food or drink (except water) for minimally 30 min prior to the visit. During study visits, participants self-administered a placebo or OXT (Syntocinon Spray; 40IU; five inhalations/insufflations per nostril) intranasally with detailed instruction and guidance from study coordinators. Intranasal doses of 24–40 IU of OXT have been administered in clinical trials 2–4 times per day without serious adverse effects and demonstrated potentially clinically meaningful effects (36, 37). Saliva was collected ∼30 min following intranasal administration to determine whether the experimental conditions had the expected effects on OXT levels. Immediately following saliva collection, participants completed a 1-h fMRI scan, then were released from the study visit. Following study visits, participants

self-administered OXT or placebo three times per day for 5 days or until menstrual onset. Participants were asked to complete a daily log documenting date and time of each intranasal dose. These logs, as well as intranasal vials containing OXT or placebo, were to be returned following each study phase. Participants also completed daily symptom reports of core emotional PMDD symptoms (described below).

Compliance

Compliance of home intranasal administration was monitored using the daily logs. Participants were marked as "compliant" if they documented completion of all doses. The study team also assessed levels of intranasal vials to determine whether or not the vials were empty or near empty to corroborate log reports.

PMDD Core Emotional Symptoms and Analyses

Throughout the entire study, participants recorded daily symptoms each evening using the DRSP. Five daily symptom outcomes were selected to examine each of the core emotional PMDD symptoms as described in DSM-5: a depression composite focused on depressed affect (mean of depression, hopelessness, and worthlessness/guilt), an anger/conflict composite (mean of anger/irritability and interpersonal conflict), a rejection sensitivity item, a mood swings item, and an anxiety item. We used composite scores (vs. single items for all domains) to reduce the number of comparisons for this study.

Daily symptoms were predicted in multilevel models in SAS PROC MIXED (METHOD=REML, DDFM=KR), with daily reports nested within women. A REPEATED statement specified an autoregressive (observation−1) structure for within-person error, and a RANDOM statement specified a random intercept and random condition contrast. Daily ratings on insufflation days during the premenstrual phase were predicted from condition (OXT days coded as 1, Placebo days coded as 0; within-person), ELA (ELA = 1, No ELA = 0; between-person), and their interaction. For significant interactions, simple slopes of OXT on daily symptoms were calculated by ELA group.

Power Considerations

Each woman contributed around 10 days of daily premenstrual symptom ratings from insufflation days (5 days per condition); there were 100 total daily observations. We conducted *post-hoc* power analyses to determine the smallest detectible effect size detectible with 80% power for both the main effect of OXT (vs. placebo) on daily symptoms and the interactive effect of ELA and OXT on daily symptoms. This N adjusted for the average clustering of symptoms within women (i.e., ICC) using the design effect calculation provided by Snijders and Bosker (38). Sensitivity analyses were conducted in G*Power to determine the smallest detectible effect size (given 80% power, alpha = 0.05, average symptom intraclass correlation (ICC) = 0.10). For the interaction among ELA and OXT, the smallest detectible effect size was $f = 0.25$. Therefore, despite the small number of women in the study, the repeated measures design and relatively low ICC of daily symptoms allowed for sufficient power to test the hypothesis that OXT would exert conventionally medium effects on daily symptoms, and that OXT and ELA would interact to exert conventionally medium-sized effects on daily symptoms. Of course, generalizability may still be limited by the small sample size.

RESULTS

Manipulation Check

Salivary OXT

For laboratory visits, salivary OXT significantly increased on OXT (vs. placebo) intranasal administration, suggesting that experimental conditions influenced OXT levels in expected directions. See **Appendix** for further information.

Home Compliance

Two out of 10 participants failed to return daily logs and intranasal vials for both premenstrual study phases; 1 out of 10 participants failed to return daily logs for only one study phase (lost in mail; she reported full completion, supported by diminished vials); and 7 out of 10 participants returned all logs and vials for both study phases. For the returned logs and vials, 100% compliance of intranasal administration was observed based on our definitions. Thus, we have confidence that most participants were compliant with study procedures.

Main and Moderated Effects of OXT on Daily PMDD Symptoms

No significant fixed main effects of OXT on any PMDD symptom were observed (p's > 0.05). However, significant random effects of OXT (vs. placebo) indicated a large degree of between-person variability in the effect of OXT on daily symptoms. Thus, the data indicated the presence of individual differences in OXT response. **Table 2** presents results of interaction tests. There were significant interactive effects of ELA (between-subject) and OXT (within-subject) on the depression composite, anger/conflict composite, anxiety, and rejection sensitivity—but not mood swings. Simple slopes for significant interactions indicated that OXT (vs. placebo) *decreased* symptoms for women without ELA, but *increased* symptoms for women with ELA (see **Table 2** for simple slopes; see **Figure 1**). Sensitivity analyses were conducted to ensure that our results were not driven by a single participant (e.g., to ensure the results were not caused by the one participant taking lisdexamfetamine, or unique psychiatric histories of various participants); systematic removal of each participant from the model did not lead to substantial changes in the pattern or significance of findings, which reduces concerns about findings being caused by a single multivariate outlier.

To provide a metric of the size of these effects, we reran simple slope models for significant interactions using person-standardized outcomes [estimated using person-specific means and standard deviations, creating a metric analogous to a within-person d; (39)]. The sizes of the OXT (vs. placebo) *difference* in symptoms among women without ELA were: 0.53 standard deviation (SD) reduction in depression, 0.86 SD reduction in anger/conflict, 0.50 SD reduction in anxiety, and 0.32 SD reduction in rejection sensitivity. The sizes of the *difference* in symptoms among women with ELA were 0.75 SD increase in

TABLE 2 | Fixed effects of ELA, oxytocin (vs. placebo), and their interactions on luteal PMDD symptoms on insufflation days.

	Daily outcomes: core emotional PMDD symptoms									
Parameter	**Depression composite**		**Anger/Conflict composite**		**Anxiety**		**Rejection sensitivity**		**Mood swings**	
	Estimate	**(SE)**	**Estimate**	**(SE)**	**Estimate**	**(SE)**	**Estimate**	**(SE)**	**Estimate**	**(SE)**
FIXED EFFECTS										
Intercept	1.58	(0.32)	2.10	(0.38)	1.96	(0.46)	1.49	(0.44)	1.72	0.37
Oxytocin (vs. Placebo)	−0.43	(0.36)	−0.84	(0.50)	−0.61	(0.51)	−0.38	(0.46)	−0.40	0.48
ELA History	0.30	(0.45)	0.10	(0.57)	0.28	(0.69)	0.96	(0.66)	0.73	0.56
ELA History × Oxytocin	**1.08***	(0.51)	**1.71***	(0.76)	**1.48***	(0.76)	**1.41***	(0.69)	0.57	0.74
SIMPLE SLOPES FOR SIGNIFICANT INTERACTIONS										
Effect of OXT in ELA	0.62	(0.47)	0.86	(0.76)	0.86	(0.79)	1.15	(0.84)		
Effect of OXT in Non-ELA	−0.43	(0.24)	−0.91	(0.35)	−0.61	(0.35)	−0.38	(0.21)		

Gamma estimates from multilevel models are analogous to unstandardized beta coefficients in OLS regression. ELA, Early Life Abuse.
**p < 0.05. Simple slope significance tests are underpowered due to small subsamples; therefore, simple slopes are descriptive only.*
The bold is to emphasize statistical significance.

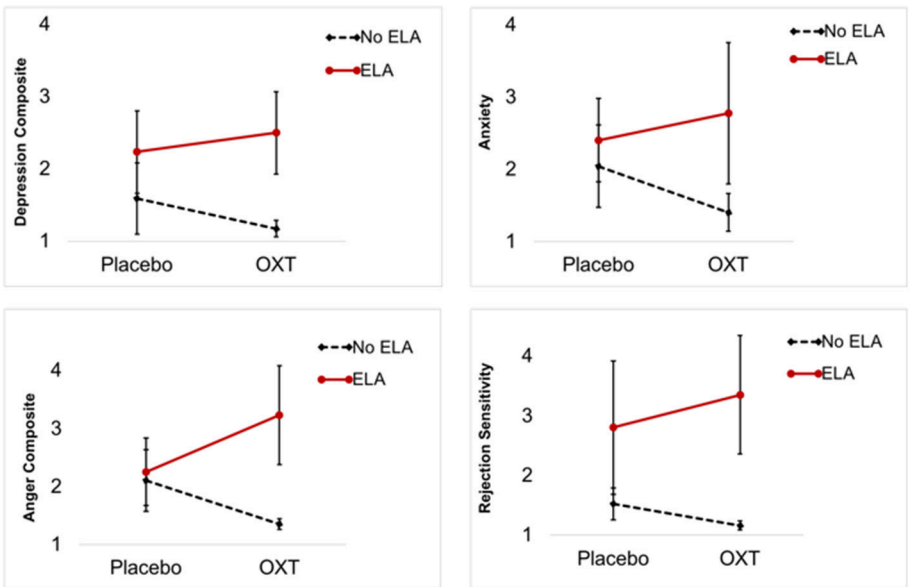

FIGURE 1 | Premenstrual symptom means and standard deviations by ELA group and experimental condition (OXT, Placebo). Each graph illustrates a significant interaction of OXT and ELA on premenstrual symptoms: OXT increased symptoms in PMDD women with ELA and decreased symptoms in PMDD women without ELA.

depression, 0.82 SD increase in anger/conflict, 0.71 SD increase in anxiety, and a 1.01 SD increase in rejection sensitivity. Given that a 1 person-SD increase is sometimes used as metric for clinically significant premenstrual symptom change, these effects would appear to suggest clinical significance. Translation of this standard-deviation based metric (within-person *d*) to *f* suggests that these interactive effects were equivalent to *f*-values ranging from 0.16 to 0.50, indicating conventionally medium-to-large effect sizes.

DISCUSSION

We found that early life abuse (ELA) moderated the impact of intranasal oxytocin (OXT) on daily emotional symptoms in a sample of women with symptoms of PMDD. Consistent with our hypotheses, intranasal OXT (vs. placebo) decreased PMDD symptoms in women without ELA, and increased PMDD symptoms in women with ELA.

To our knowledge, this is the first experimental intranasal OXT study to include daily symptom reports to prospectively predict specific patterns of OXT response based on history of ELA. Our results mirror previous studies showing intranasal OXT may not elicit prosocial responses in individuals with early adverse caregiving experiences (16–19), but extend these findings to a female-only clinical sample and includes prediction of daily symptom data during (premenstrual) OXT administration.

In this experiment, intranasal OXT had pronounced effects on social symptoms, including the anger/conflict composite and

rejection sensitivity. Some evidence points to persistent ELA-related disruptions of stress-related/neuroendocrine systems specifically in PMDD (27, 28, 30, 31) and such abnormalities may underlie the differential effects of OXT on social-emotional cognition and behavior (11). For women without ELA, OXT may normalize dysregulation of stress circuitry and/or affiliative responses [e.g., as in Social Anxiety Disorder; (14)], thereby reducing symptoms. For women with ELA, OXT may disrupt stress regulatory capacity and increase salience to social cues, which may prime threat detection and bias, and increase risk for the interpersonal symptoms that are commonly observed in affective disorders such as PMDD (6). Of note, PMDD was chosen as a model sample due to high rates of ELA and interpersonal impairment in this population; however, we do not have reason to suspect that the effects of ELA on OXT response are somehow unique to women with symptoms of PMDD.

One prior cross-sectional study has examined how early life sexual abuse in the context of PMDD (or subthreshold PMDD) moderates the association between a single premenstrual OXT measurement (via plasma) and average premenstrual symptom severity (40). The authors found that, among women with early life sexual abuse, these "tonic" premenstrual OXT levels were higher than those without early sexual abuse. Further, they found that higher tonic levels of OXT predicted a *lower* average severity of symptoms among those with early sexual abuse only. At face value this may appear inconsistent with our finding that OXT administration (vs. placebo) increased symptoms in those with ELA; however, the previous study adopted a traitlike view of premenstrual OXT (in which tonic levels were associated with symptoms), whereas our study focuses entirely on the within-person, phasic effects of placebo-controlled OXT administration. It is very possible that the tonic and phasic effects of OXT differ among those with ELA. Future work should consider this possibility, modeling both the between- and within-person effects of OXT in longitudinal and experimental studies.

Our study should be interpreted with respect to its unique strengths and weaknesses. First, our findings should be replicated in larger samples. Notably, our study was prematurely discontinued due to a facility upgrade. Despite our small sample size, the repeated measures design allowed for 80% power to test the hypothesis that ELA and OXT exert conventionally medium ($f = 0.25$) effects on daily premenstrual symptoms. Further, effect sizes for OXT-related symptom change were suggestive of medium-to-large, meaningful therapeutic effects in women without ELA and clinically-significant worsening in women with ELA. Nonetheless, these results should be interpreted with caution until they can be replicated in a larger sample. We recruited a carefully-selected clinical sample using 2 months of daily ratings to diagnose PMDD (or subthreshold PMDD) and a highly specific interview assessing abuse experiences. Although this sample is heterogeneous with respect to comorbidities, histories of abuse, and other variables, we did not observe ELA group differences in any demographic variable, and the observed heterogeneity is the norm in psychiatric and PMDD samples. Future studies should examine the unique effects of different types of abuse (e.g., emotional, physical, sexual). While the sample was primarily comprised of women meeting prospective criteria for DSM-5 PMDD, and the premenstrual mean severity was consistent with previous large PMDD samples [e.g., (41)], one individual in each ELA group demonstrated a PMDD that did not consistently show premenstrual elevation of five total symptoms (i.e., as the DSM-5 requires). Future studies should examine effects of OXT on a broader variety of PMDD symptoms. Additionally, future studies should consider including measures of biological markers, such as resting-state or task-based fMRI, to investigate neurophysiological mechanisms that are related to specific affective or social-cognitive processes following intranasal OXT administration. Such work may elucidate biological targets for evaluating novel therapeutics as a function of ELA (42). Perhaps the most notable strength of our study is the crossover experimental design, which allows for each woman to serve as her own control in a within-person test of OXT effects on symptoms. Now, larger randomized controlled trials with double-blind procedures are needed.

This study adds to an emerging literature highlighting the role of contextual and historical factors (especially those related to stress exposure) in shaping the effects of intranasal OXT on emotion and behavior in clinical samples. Although larger trials are warranted to examine the therapeutic value of intranasal OXT in PMDD and other affective disorders, the growing number of studies highlighting the potentially adverse effects of OXT in people with stress-related clinical history markers should inform the design of such studies to avoid harm and increase benefit to participants.

ETHICS STATEMENT

This research was conducted in compliance with the ethical standards of the American Psychological Association and with the approval of the University of North Carolina at Chapel Hill Institutional Review Board. Participants provided written informed consent before data collection.

AUTHOR CONTRIBUTIONS

EW developed hypotheses, conducted statistical analyses, and wrote the manuscript. TE-M conducted statistical analyses and wrote the manuscript. CP, DR, SG, and GD designed and implemented the study, and contributed to manuscript writing and editing.

FUNDING

This research was supported by the National Center for Advancing Translational Sciences, National Institutes of Health (KL2TR001109), National Center for Complementary and Integrative Health (T32AT003378), and National Institute of Mental Health (R01MH099076; T32MH093315; K99MH109667). The publication of the results from this research project was supported by the UNC-Chapel Hill ORD Publication Grant.

ACKNOWLEDGMENTS

We thank Abbey Woods and Leah Schrubbe for assistance with data collection and various aspects of this project.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00547/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 11 June 2019
doi: 10.3389/fpsyg.2019.01296

Associations Between Natural Physiological and Supraphysiological Estradiol Levels and Stress Perception

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OPEN ACCESS

Edited by:
Samantha Meltzer-Brody, University of North Carolina at Chapel Hill, United States

Reviewed by:
Angela Leigh Cumberland, RMIT University, Australia
Laverne Camille Melón, Tufts University School of Medicine, United States

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Specialty section:
This article was submitted to Psychology for Clinical Settings, a section of the journal Frontiers in Psychology

Received: *18 November 2018*
Accepted: *16 May 2019*
Published: *11 June 2019*

Citation:
Leeners B, Krüger THC, Geraedts K, Tronci E, Mancini T, Egli M, Röblitz S, Saleh L, Spanaus K, Schippert C, Zhang Y and Ille F (2019) Associations Between Natural Physiological and Supraphysiological Estradiol Levels and Stress Perception. Front. Psychol. 10:1296. doi: 10.3389/fpsyg.2019.01296

Stress is a risk factor for impaired general, mental, and reproductive health. The role of physiological and supraphysiological estradiol concentrations in stress perception and stress processing is less well understood. We, therefore, conducted a prospective observational study to investigate the association between estradiol, stress perception, and stress-related cognitive performance within serial measurements either during the natural menstrual cycle or during fertility treatment, where estradiol levels are strongly above the physiological level of a natural cycle, and consequently, represent a good model to study dose-dependent effects of estradiol. Data from 44 women receiving *in vitro* fertilization (IVF) at the Department of Reproductive Endocrinology in Zurich, Switzerland was compared to data from 88 women with measurements during their natural menstrual cycle. The German version of the Perceived Stress Questionnaire (PSQ) and the Cognitive Bias Test (CBT), in which cognitive performance is tested under time stress were used to evaluate subjective and functional aspects of stress. Estradiol levels were investigated at four different time points during the menstrual cycle and at two different time points during a fertility treatment. Cycle phases were associated with PSQ worry and cognitive bias in normally cycling women, but different phases of fertility treatment were not associated with subjectively perceived stress and stress-related cognitive bias. PSQ lack of joy and PSQ demands related to CBT in women receiving fertility treatment but not in women with a normal menstrual cycle. Only strong changes of the estradiol level during fertility treatment were weakly associated with CBT, but not with subjectively experienced stress. Our research emphasizes the multidimensional character of stress and the necessity to adjust stress research to the complex nature of stress perception and processing. Infertility is associated with an increased psychological burden in patients. However, not all phases of the process to overcome infertility do significantly increase patient stress levels. Also, research on the psychological burden of infertility should consider that stress may vary during the different phases of fertility treatment.

Clinical trial registration: ClinicalTrials.gov # NCT02098668.

Keywords: hormones, fertility treatment, menstrual cycle, stress, PSQ, cognitive bias, estrogen

INTRODUCTION

A variety of research results support an involvement of steroid hormones in the perception and processing of stress. The prevalence of affective disorders and stress-related disorders such as port-traumatic stress disorder (PTSD) is two to three times higher in women (Kessler et al., 2005; Tolin and Foa, 2006; Bromet et al., 2011; Zoladz and Diamond, 2013). These sex differences start at puberty and persist until menopause (Kessler et al., 1994; Zahn-Waxler et al., 2008).

In addition, the symptoms severity in different psychiatric diseases seems to vary with the cycle, with symptom improvement during the high-estradiol phase (Baca-Garcia et al., 2004; Davydov et al., 2005; Saunders and Hawton, 2006; Gonda et al., 2008). However, longitudinal measurements have demonstrated that in healthy women, the sex hormones do not consistently and robustly relate to negative affect or cognitive performance when assessed across two consecutive menstrual cycles (Hengartner et al., 2017; Leeners et al., 2017). In sight of such results, many of the associations interpreted as consequences of hormonal variations have to be attributed to methodological limitations.

Differences in stress system response, for example, a decreased cortisol response in the late follicular phase (Kirschbaum et al., 1999) have been reported to correlate with the menstrual cycle (Roca et al., 2005; Kajantie and Phillips, 2006). Normal ovarian hormone fluctuations seem to alter the impact of psychosocially stressful events, i.e., low estradiol levels have been reported to be associated with an increased vulnerability to psychosocial stress, for example, with an increased risk to develop PTSD (Glover et al., 2012; Albert et al., 2015). Estradiol receptors are located in brain areas that are important for the response to psychosocial stress (Love et al., 2010). In series of 12 women, brain structures involved in fear and arousal processing show attenuated responses to emotional stimuli during the late follicular phase of the menstrual cycle (Goldstein, 2005; Goldstein et al., 2010). Another small fMRI study suggested that low estradiol levels exaggerate the effect of psychosocial stress on brain activity (Albert et al., 2015); however, the small sample sizes in all three studies limit the reliability of these findings (Button et al., 2013; Turner et al., 2018). Women with higher estradiol levels appear to have less subjective distress and increased negative mood in response to a stress task when compared to women with lower estradiol levels (Albert et al., 2015).

A diagnosis of infertility is accompanied by increased stress levels, with psychological stress outweighing by far the stress associated with medical procedures (Cousineau and Domar, 2007). As stress is discussed to influence not only the success of fertility treatments but also the decision to adhere to medical support (Klonoff-Cohen et al., 2001; Klonoff-Cohen and Natarajan, 2004; Domar et al., 2015; Frederiksen et al., 2015), it is important to understand stress perception and stress processing in the context of infertility. Only few studies have investigated the effect of the estradiol level on stress perception and regulation or have compared subjective stress perception with a functional test (Holmes et al., 2002; Barker and Galea, 2010). Estradiol reaches particularly high levels at the end of a stimulation phase during fertility treatment. Therefore, this condition represents a good model to evaluate the dose-dependent effect of estradiol.

As stress seems to play a major role in women's reproductive health, it is important to better understand associations between estradiol levels and subjective as well as objective stress-related cognitive measures in the context of a natural cycle but also in the context of fertility treatment. On this background, the aim of our study was to evaluate whether (1) the subjective perception of stress and the results from a cognitive test sensitive to stress vary throughout the menstrual cycle and during the different phases of fertility treatment, (2) whether stress perception and stress-related cognitive test measurements covary, and (3) whether estradiol levels are associated with stress perception and stress-related cognitive performance.

Abbreviations: ART, Assisted reproductive techniques; CBT, Cognitive bias test; E2, Estradiol; IVF, *In Vitro* fertilization; PSQ, Perceived Stress Questionnaire.

MATERIALS AND METHODS

Study Design

We conducted a prospective observational study on the association of hormonal and psychological parameters collected within serial measurements, either during the natural menstrual cycle or during a fertility treatment. We chose to compare data from these two groups as estradiol reaches much higher levels (up to more than 10-fold) during fertility treatment than during a natural menstrual cycle, which represents a good model to investigate associations between hormonal and psychological parameters.

Study Group

Data from 44 women receiving *in vitro* fertilization (IVF) at the Department of Reproductive Endocrinology, University Hospital Zurich, Switzerland was compared to measurements during natural cycles of 88 healthy women recruited at the University Hospital Zurich and the Medical School Hannover.

The measurements were performed at key time points of a fertility treatment, e.g., the end of the downregulation/preparation phase, where hormone values are lowest and at the end of the stimulation phase where estradiol values are highest.

In women monitored during their menstrual cycle, measurements were taken at the early follicular phase, the preovulatory phase, the midluteal phase, and shortly prior to expected menses. Further details of hormone measurements in women during their natural menstrual cycle have been previously reported (Hengartner et al., 2017; Leeners et al., 2017). Results of cognitive performance throughout two consecutive cycles have been described earlier (Leeners et al., 2017).

Women undergoing fertility treatments were subjected to the normal investigation of fertility disorders at the Department of Reproductive Endocrinology, University Hospital Zurich, Switzerland. A gynecological examination and transvaginal ultrasound were performed to determine the antral follicle count and uterine or adnexal abnormalities. The evaluation of hormones (LH, FSH, estradiol, anti-Mullerian hormone; testosterone, 17 hydroxyprogesterone, prolactin, thyroid

stimulating hormone on specific indication) in the early follicular phase (day 2–5) served to investigate endocrinological disorders. A semen analyses was conducted in the male partners. Depending on the result of the semen analysis and eventual urological treatment, a hydrosonography of the uterine cavity, a hydro-contrast-sonography or a hysterosalpingography were performed to evaluate uterine and/or tubal pathology. Hepatitis B, C, HIV, and chlamydia infection was investigated in both partners. Within the initial patients' history, relevant inclusion and exclusion criteria were evaluated, for example, linguistic capacity or medical conditions, which might influence cognitive performance such as psychiatric diseases. Premenstrual syndrome was also an exclusion criterion for study participation.

Measurements of Stress

Perceived Stress Questionnaire

The Perceived Stress Questionnaire (PSQ) (Fliege et al., 2005) conceptualized by Levenstein et al. (1993) was used to evaluate subjectively perceived stress. The German version of the PSQ has been validated in a German sample (N = 650) (Fliege et al., 2001). In contrast to the original PSQ, only the factors worry, tension, joy, and demands could be confirmed. These scales are composed of five items each resulting in a total of 20 items that show good internal consistency with Cronbach's α between 0.80 and 0.86. The scales "worries," "tension," and "joy" are estimated to represent the internal stress reaction, while the variable "demands" is considered to represent experiences toward external stressors. Construct validity has been evaluated by investigating associations with subjectively perceived quality of life as measured with the World Health Organization Quality of Life Questionnaires in its short-version (WHOQOL-Bref) and with social support as measured with a German questionnaire to evaluate social support ("Fragebogen zur Sozialen Unterstützung," F-SOZU). A strong association with results from both questionnaires confirms high construct validity. External validity was supported by results from psychosomatic patients prior to therapy, women after abortion, and women after normal delivery. The German version of the PSQ seems to be sensitive to longitudinal changes of stress experiences as has been demonstrated by decreasing stress values during in-patients psychotherapy (Fliege et al., 2001). For each item, the study participants had to choose the most appropriate answer for the last 24 h between the options "rarely," "sometimes," "often," and "mostly," i.e., a numerical scale ranging from 1 to 4, where one is the least and 4 is the most.

Cognitive Bias Test

The Cognitive Bias Test (CBT) is a multiple choice procedure designed by Goldberg et al. (1994) as a bias (preference) to evaluate complex cognitive functions. The CBT entails designs characterized along five binary dimensions: shape (circle/square), color (red/blue), number (one/two identical components), size (large/small), and contour (outline/filled with a homogeneous color). Study participants have to rate similarity between two items. The items are on different levels of difficulty and presented twice in different vertical positions to the study participant. Thus, 32 stimuli can be generated, and a "similarity index" computed between any two stimuli, ranging from 5 (identical) to 0 (differing along all five dimensions). The "similarity indices" between targets and subject's choices are summed across trials (Goldberg et al., 1994). In the present study, we used correct responses as the outcome, that is, higher scores on the CBT indicate better cognitive control. The decisions have to be taken under time stress, that is, after few seconds, noises and blinking color signals increase the pressure to take a decision. On this background, the cognitive bias test is considered to not only capture cognitive performance, but also functional reactions toward stress (Lehner et al., 1997; Yu, 2016).

Ethics

This study followed the guidelines of the World Medical Association Declaration of Helsinki 1964, updated in October 2013 and was conducted after approval by the Cantonal Committee of Zürich, Switzerland. All participants provided a written informed consent for study participation. Women were compensated for their expenditures associated with the study participation. The study has been registered in clin.trial.gov (NCT02098668).

Statistics

Estradiol levels were log-transformed for statistical analysis. Repeated measures of PSQ and CBT were examined using Generalized Estimating Equations (GEE). These statistical models were introduced to fit regression analyses that account for within-subject correlation, which is an inherent part of longitudinal studies that rely on repeated outcome measures (Zeger et al., 1988). GEE are considered state-of-the-art for longitudinal data analysis and superior to repeated measures ANOVA due to their psychometric properties (Ballinger, 2004; Gibbons et al., 2010). GEE use all available data and impute missing values under the assumption of Missing Completely at Random (MCAR). Because all PSQ dimensions and CBT scores were right skewed interval scales, we fitted all models with Gamma distribution and log link-function. The within-subject covariance was specified with the "unstructured" correlation type to avoid having any constraints on the covariance structure and a robust sandwich estimator was used to reduce the effects of outliers and influential observations. All analyses were conducted with SPSS version 24.

RESULTS

Altogether, 85 women were investigated during their natural menstrual cycle and 44 during a fertility treatment. Mean age in the first group was 30.2 ± 5.5 years (range 20–43) and 36.0 ± 3.4 years (range 28–44; $p < 0.001$) in women undergoing fertility treatment. In 13 women, fertility treatment was performed because of a mechanical problem, in 14 because of endometriosis in 10 because of polycystic ovary syndrome (PCOS), in 6 because of idiopathic sterility and in 34 women either because of male factor only or a reduced sperm quality in addition

to the listed female indications. Some of the couples had several causes of infertility.

No differences in baseline estradiol levels between women in their natural cycle and women in fertility treatment nor between women receiving fertility treatment because of female indication only, combined male and female indication or male indication only was found.

Variation of Perceived Stress Questionnaire and Cognitive Bias Test Across Time

PSQ worry varied significantly across the menstrual cycle ($p = 0.001$). The mean scores declined steadily across measurement occasion. A linear time trend was statistically confirmed using a polynomial contrast analysis (contrast estimate: −0.774, SE = 0.193; Wald $\chi^2 = 16.069$, df = 1, $p < 0.001$). PSQ tension, PSQ lack of joy, and PSQ demands remained stable across the cycle, whereas for cognitive bias, there was again evidence of significant change ($p = 0.004$). On a descriptive level, cognitive bias was highest during menstrual phase, declined markedly at pre-ovulatory phase and reached an intermediate plateau across both mid-luteal and pre-menstrual phase. According to a polynomial contrast analysis, the time trend was quadratic (contrast estimate: 0.623, SE = 0.224, Wald $\chi^2 = 7.722$, df = 1, $p = 0.016$). In women receiving fertility treatment, all PSQ scales as well as cognitive bias remained stable across time. Comparing the PSQ scales and cognitive bias between groups (controls versus fertility treatment) revealed no significant differences, as all confidence intervals overlapped. That is, control women and women receiving fertility treatment did not differ in both their perceived stress and cognitive bias.

Covariation Between Perceived Stress Questionnaire and Cognitive Bias Test

Next, we examined whether perceived stress and cognitive bias covary across time. As indicated in **Table 1**, in the control group, PSQ scales were not associated with cognitive bias, indicating that, inter-individually, subjectively perceived stress

TABLE 1 | Associations of perceived stress with cognitive bias across time.

	Cognitive bias		
	B	**95% CI**	***p****
PSQ worry			
Control	0.009	−0.003; 0.020	0.137
Fertility treatment	−0.010	−0.020; 0.000	0.053
PSQ tension			
Control	0.006	−0.005; 0.017	0.250
Fertility treatment	−0.005	−0.015; 0.005	0.333
PSQ lack of joy			
Control	0.003	−0.006; 0.013	0.499
Fertility treatment	−0.017	−0.030; −0.004	0.009
PSQ demands			
Control	0.008	−0.001; 0.018	0.070
Fertility treatment	−0.020	−0.031; −0.009	0.001

**Adjusted α = 0.01.*

TABLE 2 | Perceived stress and cognitive bias across time.

	Measurement occasion				Model effect
Measurements during menstrual cycle	**T1 Menstrual phase**	**T2 Pre-ovulatory phase**	**T3 Mid-luteal phase**	**T4 Premenstrual phase**	
Measurements during fertility treatment	**End of down-regulation/preparation phase**	**End of stimulation phase**			
	Mean (95% CI)	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	***p***
PSQ worry					
Control	9.7 (8.9–10.5)	9.1 (8.4–9.9)	8.9 (8.1–9.6)	8.6 (7.9–9.3)	0.001
Fertility treatment	8.9 (8.0–9.8)	8.8 (7.9–9.8)			0.809
PSQ tension					
Control	11.4 (10.7–12.1)	11.1 (10.4–11.8)	11.1 (10.5–11.8)	11.3 (10.6–12.0)	0.694
Fertility treatment	11.2 (10.2–12.2)	11.2 (10.2–12.4)			0.860
PSQ lack of joy					
Control	9.9 (9.3–10.6)	10.2 (9.5–10.9)	10.2 (9.6–10.9)	10.3 (9.6–11.0)	0.574
Fertility treatment	10.3 (9.5–11.2)	10.1 (9.1–11.1)			0.645
PSQ demands					
Control	11.7 (11.1–12.4)	11.0 (10.4–11.7)	10.8 (10.1–11.6)	11.1 (10.4–11.7)	0.093
Fertility treatment	10.3 (9.5–11.2)	10.4 (9.3–11.6)			0.831
Cognitive bias					
Control	7.4 (6.7–8.1)	6.2 (5.6–6.8)	6.5 (5.8–7.2)	6.5 (5.8–7.3)	0.004
Fertility treatment	7.1 (6.2–8.1)	7.2 (5.7–9.0)			0.902
Estradiol (log)					
Control	5.0 (4.9–5.1)	6.4 (6.3–6.6)	6.3 (6.2–6.4)	5.7 (5.5–5.8)	<0.001
Fertility treatment	3.4 (3.1–3.8)	8.2 (8.0–8.5)			<0.001

TABLE 3 | Associations between E2 and stress.

	E2 levels		
	B	95% CI	*p**
PSQ worry			
Control	–0.009	–0.056; 0.039	0.723
Fertility treatment	–0.008	–0.058; 0.043	0.767
PSQ tension			
Control	–0.023	–0.062; 0.015	0.236
Fertility treatment	0.018	–0.034; 0.071	0.491
PSQ lack of joy			
Control	0.003	–0.035; 0.042	0.864
Fertility treatment	0.015	–0.041; 0.071	0.603
PSQ demands			
Control	–0.054	–0.100; –0.008	0.021
Fertility treatment	0.018	–0.048; 0.083	0.600
Cognitive bias			
Control	–0.053	–0.123; 0.016	0.132
Fertility treatment	0.232	0.120; 0.345	<0.001

**Adjusted α = 0.01.*

does not correlate with cognitive bias. In contrast, in women receiving fertility treatment, there was a small negative association with PSQ lack of joy and PSQ demands. These findings indicate that, women who report both increased lack of joy and demands, have lower cognitive bias scores. Both effect sizes corresponded to approximately Pearson $r = -0.2$.

Covariation Between Perceived Stress Questionnaire, Cognitive Bias Test, and Estradiol

The log-transformed estradiol levels at different time points are presented in **Table 2**. The repeated associations of estradiol with PSQ scales and cognitive bias are indicated in **Table 3**. In the control group, there was a negative association between PSQ demands and estradiol levels corresponding to an effect size of approximately Pearson $r = -0.1$. That is, women who had higher estradiol levels over time reported slightly lower demands. However, this association did not reach statistical significance when adjusted for multiple testing (adjusted $\alpha = 0.01$). In women receiving fertility treatment, estradiol was positively associated with cognitive bias, indicating that women with higher estradiol levels experienced more cognitive bias. The effect size corresponded to Pearson $r = 0.2$ and remained statistically significant after adjusting for multiple testing.

DISCUSSION

During the natural cycle, worries exhibit higher values during the menstrual period, and to a lesser degree during the pre-ovulatory phase. Experiences of tension, lack of joy and demands do not change with cycle phases. Also, in women receiving fertility treatment, no differences between various aspects of perceived stress could be demonstrated between the end of the downregulation/preparation phase and the end of the stimulation phase, where estradiol values are highest. In cycling women, stress-related cognitive bias was highest during the menstrual phase, while no differences were detected in the evaluated phases of a fertility treatment. During the natural cycle, no significant associations between estradiol levels and scores in the PSQ or the CBT could be demonstrated. In contrast, higher levels of estradiol as in fertility treatments were associated with increased stress-related cognitive bias; however, the effect size was rather small.

Interestingly, no significant differences in the perceived stress level could be demonstrated between women in their natural cycle and women receiving fertility treatment. Also, results in the PSQ as representing subjectively experienced stress and results from the CBT as a functional measurement did not correlate in women with a natural cycle. However, women undergoing fertility treatment presented a lower stress-related cognitive bias when lack of joy and demands as measured by the PSQ were increased.

Does Stress Perception and Stress-Related Cognitive Bias Change With Cycle Phases/Fertility Treatment?

During the natural cycle, significant changes in three different stress dimensions were found. While worries were higher during the pre-ovulatory phase and highest during the menstrual phase, tension, lack of joy and demands were stable throughout the cycle. As these differences are not related to estradiol levels and levels of other hormones such as progesterone or testosterone, which only differ very slightly between the menstrual and the preovulatory phase, it is not possible to provide mechanistic explanations for our findings. The lack of estradiol-related changes is also confirmed by the missing differences between various aspects of perceived stress between the end of the downregulation/preparation phase and the end of the stimulation phase, where estradiol values are highest, in women receiving fertility treatment. On this background, physical symptoms such as the bleeding or dysmenorrhea, that is, pain related to the menstrual bleeding, are likely to be responsible for differences in findings. Unfortunately, we did not evaluate any details on menstrual bleeding, for example, if heavy bleeding or pain might have increased worries during this period. As the women investigated during their menstrual cycle were not aiming for a pregnancy, frustration about the bleeding is also unlikely to explain our findings.

Also, during the menstrual phase, stress-related cognitive bias was highest, while no differences were detected in different phases of a fertility treatment. Although, it would be plausible that increased worries during the menstrual phase interfere with cognitive performance, our results on stress perception showed no association with cognitive performance. Previous research has demonstrated that cycle-related differences are not consistently found across several consecutive cycles (Leeners et al., 2017). Also, the lack of differences during the two time points of fertility treatment supports that changes in the estradiol levels alone are unlikely to induce changes in stress-related cognitive performance. Our findings are in contrast with previous

results, which showed an association between estradiol and stress perception (Goldstein, 2005; Goldstein et al., 2010; Albert et al., 2015); however, due to the small sample sizes the reliability of these fMRI studies is questionable (Button et al., 2013; Turner et al., 2018). In addition to the above-mentioned misinterpretation of findings, dissimilarities in stress measurement may also account for the differences. Also, methodological aspects, for example, learning effects or the phenomenon of "Regression to the mean" might have influenced absolute test values and might consequently have reduced differences between consecutive measurements. While several available studies focus on the role of stress in the onset of psychiatric disease (Glover et al., 2012; Zoladz and Diamond, 2013), our study investigates subjective stress perception and stress-related cognitive bias.

Are Subjectively Perceived Stress and Stress-Related Cognitive Bias Measures Different in Women During Their Menstrual Cycle and During Fertility Treatment?

Although infertility is well-known to be associated with stress (Cousineau and Domar, 2007; Gameiro et al., 2013; Rockliff et al., 2014; Pasch et al., 2016), our findings show no significant differences in the perceived stress level as well as the cognitive bias test between women receiving fertility treatment and women in their natural cycle. While the later measurements of the CBT may be influenced though learning effects and the phenomenon of "Regression to the mean," these effects cannot explain the lack of differences in the first measurement of the series. About 15–20% of the couples receiving ART need psychosocial support (Boivin, 2002); however, the great majority succeeds to face diagnosis and treatment of infertility without additional support. This notion is sustained by our findings. Previous research has shown that although specific psychological factors associated with infertility-related distress can be identified, there are strong individual differences (Rockliff et al., 2014). Also, the concepts to provide psychological support in different centers offering fertility medicine vary greatly. In our department, two fertility specialists with a psychological education provide support for couples when needed and wanted. The lack of increased stress in the women investigated in the present study may also be explained by the fact, that infertile women experience psychological stressors as stronger burden than medical procedures such as oocyte pick-up, collection of blood samples, or anesthesia (van Balen et al., 1996; Hammarberg et al., 2001). The feeling of helplessness and waiting times, that is, phases where nothing can be done to improve chances for pregnancy are particularly stressful. While differences between women in their natural cycle and infertile women might be greater in the context of a diagnosis of infertility, shortly after treatment failure or during the often prolonged waiting times, our results have been collected during fertility treatment, that is, a very active phase, where the possibility of a pregnancy is experienced as high and positive feelings might consequently be stronger than in other phases. During this period, women are, moreover, intensively supported by doctors and nurse. This support in combination with the fact that IVF is associated with the best available chances to achieve a pregnancy may help to reduce the stress levels that are generally associated with infertility. Reduction of psychological burden in phases of high frequency contacts with health care providers has also been reported in the context of other diseases (Leeners et al., 2008). Our results on the subjective perception of stress as well as in the CBT as an objective test, support the notion that the medical procedures are not associated with increased stress levels. Also, the increasingly open discussion about infertility, along with an increasing easiness to receive support from women or couples in similar situations through the internet and a rising awareness that psychological support is an important component in successful fertility treatment, might help to reduce infertility-related stress (Boivin, 2002; Gameiro et al., 2013).

Is There an Association Between Stress Perception and Stress-Related Cognitive Bias?

The subjectively experienced stress and stress-related cognitive bias did not correlate in women with a natural cycle. Research on stress is challenged by the absence of objective criteria to measure individual stress (Nesse et al., 2010). A lack of correlation between subjective and objective stress measures has been previously described and might also explain the lack of correlation between our PSQ and CBT findings. Nonetheless, our results confirm that stress is multidimensional and stress research should consequently combine subjective and objective as well as functional tests to collect the full picture of stress experience in study participants.

Interestingly, women undergoing fertility treatment presented a lower cognitive bias when lack of joy and demands as measured by the PSQ were increased. While we have no explanation for why the lack of joy is associated with decreased cognitive bias, adapting to higher demands might result in increased concentration, and consequently allows to reduce cognitive bias.

Is There Any Association Between Estradiol Levels and Stress Perception or the Cognitive Bias?

Currently, the role of estrogen on the response to psychosocial stress is only partly understood. In our study, higher levels of estradiol, as occurring in fertility treatments, were associated with increased cognitive bias; however, the effect size was minute. In contrast, no significant associations between estradiol levels and stress as represented by the scores in the PSQ or the CBT could be demonstrated during the natural cycle. A previous study showed that even though small changes of cognitive parameters in relation to hormonal changes can be detected in a first cycle, a comparison with a consecutive cycle shows that such changes cannot be reliably reproduced (Leeners et al., 2017). Therefore, we expect that only strongly elevated estradiol levels, as occurring in a fertility treatment, may exhibit an association with cognitive bias. A dose-dependent effect of E2 has also been described in other studies (Holmes et al., 2002; Barker and Galea, 2010). However, as

stress-related cognitive performance did not differ significantly between the two time points of fertility treatment, the association between strongly elevated estradiol levels and CBT results may hint at a lack of clinical relevance. An analysis of 259 women throughout two menstrual cycles showed an association between estradiol levels and stress (Wactawski-Wende et al., 2009; Schliep et al., 2015). However, although the actual desire for a pregnancy and known gynecological problems were exclusion factors for study participation, the suspicion of a fertility problem as a motivating factor to participate in the study was not taken in consideration. As women with reproductive dysfunction showed higher stress levels and low estradiol levels may be the result of impaired follicular maturation, the association between estradiol levels and stress levels might eventually have been biased by fertility problems.

Previous studies support the role of ovarian hormones as a mediating factor for the effect of psychosocial stress (Glover et al., 2012; Albert et al., 2015). Estrogen has been shown, for example, to modulate the brain response to negatively valanced images or negative emotional information (Goldstein, 2005; Andreano and Cahill, 2010; Merz et al., 2012). As the evaluated cognitive test represents reactions to time stress, our findings for high estradiol levels seem to support such a role. While previous research has demonstrated associations between high estradiol levels and an attenuation of negative mood response to psychosocial stress, that is, increased vulnerability in phases of low estradiol levels (Albert et al., 2015), our results show increased cognitive bias when estradiol levels are very high. As estradiol has been described to attenuate sympathetic and HPA axis activity to stress (Putnam et al., 2005; Kajantie and Phillips, 2006), these findings cannot be explained by an effect of estradiol on the systemic stress reaction. Other research demonstrated an association between high estradiol levels with greater hippocampal activity during psychosocial stress in normally cycling premenopausal women (Albert et al., 2015). It is possible that such activities to process stress, that is, increased brain activities in certain regions interfere with cognitive performance. Estradiol receptors are located in a number of brain areas, including but not limited to regions important for the autonomic, hormonal, and cognitive-emotional response to psychosocial stress (Love et al., 2010). Therefore, it is difficult to evaluate the complex interactions between estradiol and stress processing with their impact on cognitive bias. As heart rate responses to stress seem to be greater at high estradiol levels, which are in line with our findings (Kirschbaum et al., 1996), estradiol seems to have opposite effects on different physiological stress reactions.

Strength and Limitations

A great strength of this study is the combination of serial hormonal measurements with a questionnaire evaluating subjectively perceived stress and with stress-related cognitive bias. A comparison of data collected in natural cycles to those collected in fertility treatments in which estradiol levels increase far beyond the cyclic maximum represents a natural model to evaluate associations between stress and high estradiol levels.

However, important methodological aspects question the reliability of the associations described above. First, the association between self-reported measures and hormonal values is subject to random fluctuation. In contrast to the subjective stress measure, cognitive bias as a systematic functional evaluation is likely to be more reliable but may be influenced through learning effects. Also, the phenomenon of "Regression to the Mean" (Senn, 2011) will likely result in a steady reduction of extreme values, so that the last measurements in a series may show smaller differences than the first measurements. A strong correlation between changes in CBT between the two time points and the CBT results in the first measurement ($r = 0.35$) may indicate such a "Regression to the mean." In regard to the two aforementioned limitations, we cannot fully exclude a complex interaction of limitations and factual differences.

Self-report questionnaires as the PSQ in our study may under-report the true level of distress since patients may feign emotional well-being because of social desirability or to appear psychologically healthy toward fertility specialists. However, our study participants were currently undergoing fertility treatment, that is, the decision to initiate treatment was already taken, so that such bias can be excluded. As perceived stress does not necessarily correlate to stress hormone levels (Faresjö et al., 2013; Gerber et al., 2013) it would have been beneficial to also add such stress measures to our study. Although, any woman receiving fertility treatment and sufficiently mastering the German language was invited for study participation, selection bias because of women feeling sufficiently relaxed to agree to the additional burden of study participation cannot be excluded. However, this additional burden was also relevant for women during their menstrual cycle.

The focus of our research was to evaluate associations between estradiol levels and stress. Therefore, we included women during fertility treatments irrespective of whether they were in a first or consecutive treatment cycle. Although stressors in first and later treatment cycles may be different, for example, fear of side effects in a first treatment cycle and fear of treatment failure in a later cycle, such difference should not influence our results as we evaluate associations between estradiol levels and stress or associations between perceived stress and cognitive bias. Future work should differentiate between stress related to the fertility treatment and other stress at each timepoint.

Estradiol-related effects are part of a complex network determining experiences and reactions to psychosocial stress in women; future studies should not only include further hormones such as progesterone or testosterone but also life-time positive and negative eventually traumatic experiences, personality factors, physical and mental diseases or chronic stress (Fliege et al., 2001) as well as physical and psychological factors related to the actual menstrual cycle.

CONCLUSION

The presented findings support the notion that only some aspects of subjective stress perception correlate with stress-related functions. Although infertility is known to be associated with increased

psychological burden, such stress seems not to be present in all phases of fertility treatment. Cycle phases correlate only in some parameters and different phases of fertility treatment do not correlate with subjectively perceived stress and stress-related cognitive bias. Only strong changes of estradiol levels were weakly associated with stress-related cognitive bias, but not with subjective experienced stress. While methodological aspects such as learning effects potentially resulting in reduction of cognitive bias or the phenomenon of "Regression to the mean" may limit the impact of the presented findings, our research emphasizes the multidimensional character of stress, and the necessity to adjust stress research to the complex nature of stress perception and processing. Also, research on the psychological burden of infertility should take into consideration that stress may vary during the different phases of fertility treatment.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Swiss regimentation for studies involving human subjects as approved by the Cantonal ethics committee of Zurich, Switzerland with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Cantonal ethics committee of Zurich.

AUTHOR CONTRIBUTIONS

BL, TK, and FI designed this study. BL, TK, CS, and YZ collected data. LS and KS performed hormonal analysis. KG and FI organized the database. BL and FI analyzed the data. BL wrote the first draft of the manuscript. TK and FI improved the first version of the manuscript. ET, TM, and ME critically revised the first version of the manuscript. All authors contributed to manuscript conception, manuscript revision, read and approved the submitted version.

FUNDING

The evaluation of the hormonal parameters was supported by a FP-7 European grant (PAEON 600773) and the Bangerter-Rhyner Foundation. The sponsors had no further role in the experimental design, the collection, analysis, and interpretation of data, the writing of this report, or the decision to submit this paper for publication.

ACKNOWLEDGMENTS

We gratefully acknowledge all study participants for supporting our research.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 11 January 2019
doi: 10.3389/fpsyg.2018.02767

Negative Association Between Allopregnanolone and Cerebral Serotonin Transporter Binding in Healthy Women of Fertile Age

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Edited by:
Sophie Schweizer, Universität Heidelberg, Germany

Reviewed by:
Angela Kuhla, Universitätsmedizin Rostock, Germany
Angela Leigh Cumberland, RMIT University, Australia

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Specialty section:
This article was submitted to Clinical and Health Psychology, a section of the journal Frontiers in Psychology

Received: *24 September 2018*
Accepted: *24 December 2018*
Published: *11 January 2019*

Citation:
Sundström Poromaa I, Comasco E, Bäckström T, Bixo M, Jensen P and Frokjaer VG (2019) Negative Association Between Allopregnanolone and Cerebral Serotonin Transporter Binding in Healthy Women of Fertile Age. Front. Psychol. 9:2767. doi: 10.3389/fpsyg.2018.02767

Allopregnanolone is a metabolite of the sex hormone progesterone, with suggested relevance for female mood disorders. While allopregnanolone and serotonin are known to influence psychological well-being, the molecular and psychological specifics of their relationship are to date poorly understood, especially in women of fertile age who experience regular fluctuations of progesterone across the menstrual cycle. Availability of serotonin in the synaptic cleft is regulated by the serotonin transporter (SERT), which can be imaged in the living human brain by use of positron emission tomography (PET) and the radiotracer [^{11}C]DASB. To evaluate sex-specific allopregnanolone-SERT interactions, the present study investigated the relationship between cerebral SERT availability, serum allopregnanolone levels and psychological well-being in women of fertile age. Brain imaging data, self-reported symptoms of mental distress and emotion regulation, and biobank material from ninety healthy women were available from the Center for Integrated Molecular Brain Imaging (CIMBI) database. Age, BMI, and daylight minutes were included as covariates in the analyses and SERT genotype (5-HTTLPR) was considered a potential confounder. Lower serum allopregnanolone levels were associated with higher SERT binding in the prefrontal cortex. Moreover, allopregnanolone levels were negatively associated with measures of alertness, although this finding was not mediated by prefrontal cortex SERT binding. These findings suggest a link between the typical psychological well-being experienced in the follicular phase when allopregnanolone levels are low and higher SERT in the prefrontal cortex, a region for higher cognitive functions and top-down regulation of emotions.

Keywords: allopregnanolone, brain, mood, PET, serotonin transporter, women, 5HTT

INTRODUCTION

Progesterone is, together with estradiol, one of the ovarian steroid hormones. Progesterone fluctuations are pronounced during the menstrual cycle, but even more extreme variations are noted across pregnancy and postpartum. Increasing evidence suggest that progesterone influences emotion processing in healthy, naturally cycling women (Toffoletto et al., 2014; Comasco and Sundstrom-Poromaa, 2015). So far, earlier work supports worsened emotion recognition, enhanced emotional memory and increased amygdala reactivity at times of luteal phase progesterone levels (van Wingen et al., 2008; Sundstrom Poromaa and Gingnell, 2014). Furthermore, progesterone has been implicated as a causal agent for premenstrual syndrome and premenstrual dysphoric disorder (PMDD) (Sundstrom et al., 1999; Segebladh et al., 2009). However, while it may be assumed that some of these effects are mediated via the progesterone receptor, some of the typical progesterone-induced mood symptoms could equally well be mediated by γ-aminobutyric acid (GABA)-active progesterone metabolites, such as allopregnanolone (Backstrom et al., 2012). Of relevance, allopregnanolone serum concentrations temporarily follow that of progesterone during the menstrual cycle, but with an off-set of one to two days (Ottander et al., 2005). Further, whereas progesterone levels increase by 25-fold during the luteal phase, allopregnanolone levels vary by approximately four-fold (Nyberg et al., 2007).

Animal models point to sedative, anxiolytic, anti-convulsant, and neuroprotective properties of allopregnanolone (Kask et al., 2008; Melcangi et al., 2011; Brunton, 2015). In humans, a distinct role of allopregnanolone for female mood disorders has been difficult to delineate, and at present findings point in two directions. First, in pregnant women low levels of allopregnanolone have been associated with depressive symptoms (Hellgren et al., 2014, 2017), and a proof-of-concept study recently demonstrated that allopregnanolone infusion in the postpartum period was able to rapidly alleviate postpartum depressive symptoms (Kanes et al., 2017). On the other hand, in terms of the menstrual cycle-induced mental health problems, the allopregnanolone antagonist sepranolone was recently demonstrated to hold promise as treatment of PMDD (Bixo et al., 2017). These disparities in effect may be explained by differences in underlying pathophysiology, but potentially also by a suggested inverted U-shaped relationship between allopregnanolone and psychological wellbeing (Backstrom et al., 2014). The relationship between allopregnanolone and serotonergic neurotransmission has received little attention in humans, but animal studies have shown that acute administration of fluoxetine increases allopregnanolone levels in the brain of female rats (Fry et al., 2014; Devall et al., 2015) and diminishes their sensitivity to stress (Devall et al., 2015). Clearly, further studies are needed to elucidate the role of allopregnanolone in female mood disorders, and its' relationship with the serotonin system.

Recently, menstrual cycle phase-dependent associations between allopregnanolone and fMRI resting state functional connectivity was demonstrated in healthy women (Syan et al., 2017). In the mid-follicular phase, allopregnanolone levels correlated negatively with resting state functional connectivity between the seed region of the default mode network, the posterior cingulate cortex, and the somatosensory association cortex in the sensorimotor network. In the late luteal phase, among other associations, allopregnanolone levels correlated negatively with connectivity between another region of the default mode network, the medial prefrontal cortex (mPFC), and primary and association visual cortices, but positively with connectivity between the mPFC and the entorhinal cortex in the limbic system (Syan et al., 2017). Moreover, a one-subject longitudinal study found progesterone-dependent modulation of resting state connectivity between the hippocampus and dorsolateral prefrontal cortex (Arelin et al., 2015). Thus, the primary brain regions of choice for this study were the midbrain, pallidostriatum and prefrontal cortex, all important representatives of serotonergic neurotransmission, and amygdala, insula, posterior cingulate and hippocampus for their association to emotion processing and allopregnanolone or progesterone-influenced connectivity.

In terms of molecular imaging of markers of serotonergic neurotransmission, no study to our knowledge has investigated serotonin transporter (SERT) binding in relation to allopregnanolone. Thus, the present study sought to investigate cerebral SERT binding in relation to allopregnanolone serum levels in women of childbearing age. Allopregnanolone serum concentration was expected to negatively associate with SERT availability, and also to influence measures of mood.

MATERIALS AND METHODS

Subjects

Healthy women (age < 50 years) with regular menstrual cycle (23–35 days), BMI < 50 kg/m^2, and no use of hormonal contraception or therapy, who had undergone PET scanning on a high resolution research tomograph (HRRT) and had a serum sample from the day of the PET scan stored in the CIMBI Biobank (Knudsen et al., 2016) were included in the study. With these criteria, serum samples of 92 women who had undergone [^{11}C]DASB scan were available for the study. Two blood samples could not be analyzed for technical reasons, leaving a study population of 90 healthy women for the [^{11}C]DASB analyses.

Besides exclusion criteria for neuroimaging, we excluded subjects with clinically relevant medical history, such as neurological or psychiatric disorders, and history of severe head trauma, drug or alcohol abuse, or clinically relevant findings on routine blood chemistry were excluded. All participants had normal findings on brain magnetic resonance imaging (MRI) and displayed no psychopathology according to the revised symptom checklist (SCL-90-R) (Derogatis and Savitz, 1999) and the major depression inventory (MDI). The participants have previously been included as healthy volunteers in studies on GnRH agonist treatment (Frokjaer et al., 2015; Macoveanu et al., 2016;

Fisher et al., 2017a), serotonin transporter binding (Fisher et al., 2017b), gastric bypass surgery (Haahr et al., 2015), and functional connectivity (Beliveau et al., 2015). All projects were approved by the Copenhagen Region Ethics Committee (KF-01-2006-20, KF-01-124/04, H-1-2010-085, and H-2-2010-108), and the women provided written informed consent for participation.

Allopregnanolone Analyses

Blood samples were collected on the day of the PET scan. The blood samples were centrifuged immediately after the phlebotomy and plasma was stored at −20°C. Allopregnanolone plasma concentrations were determined by Umeå Neurosteroid Research Center, in one batch, as previously described (Timby et al., 2006). For measurement of allopregnanolone, we used radioimmunoassay (RIA) after a first step involving extraction with diethyl ether and purification by celite chromatography, the latter to reduce cross-reactivity. The antibody used in the RIA had been raised against 3α-hydroxy-20-oxo-5α-pregnan-11-yl carboxymethyl ether coupled with bovine serum albumin (AgriSera AB, Umeå, Sweden). A RackBeta (Wallace, Finland) scintillation counter was used to count the samples. The allopregnanolone detection limit was 25 pg/ml, with intraassay coefficient of variation for of 6.5% and an interassay coefficient of variation of 8.5%.

SERT Imaging

SERT binding was imaged with [^{11}C]DASB-PET during a 90- min dynamic acquisition directly following the bolus injection of the tracer. A Siemens ECAT HRRT scanner (Siemens, Munich, Germany), operating in three-dimensional acquisition mode, with an in-plane resolution of 2 mm, was used to obtain the PET scans. The outcome of the [^{11}C]DASB binding was the ratio between specific binding and non-displaceable binding of the tracer. We used a modified reference tissue model, using cerebellum as a reference region (the vermis part excluded), designed to quantify [^{11}C]DASB (multilinear reference tissue model/multilinear reference tissue model 2) (Ichise et al., 2003), and implemented in PMOD (version 2.9; PMOD Technologies, Zurich, Switzerland). Movement correction was performed by AIR (Woods et al., 1992). The co-registration of the [^{11}C]DASB mean image to the high-resolution T1-weighted MR image was done using SPM8 (Ashburner and Friston, 1997). The exact details on the [^{11}C] DASB imaging has been presented previously (Frokjaer et al., 2009).

Volumes of Interest

Volumes of interest for this study were outlined on the participant's MRIs, as described previously (Svarer et al., 2005). In order to constrain the number of statistical comparisons we pooled a number of regions. This approach was based on the observation of high correlation of SERT binding between cortical and high-binding subcortical regions (Erritzoe et al., 2010). Thus, an average SERT binding potential was computed for each participant for the prefrontal cortex (computed by pooling orbito-, superior-, and medial- and inferior-frontal cortex), the pallidostriatum (a combined subcortical region), and the midbrain (including the raphe nuclei). These three VOIs were the primary outcomes of the study. In addition, four regions served as secondary VOIs; amygdala, insula, hippocampus, and posterior cingulate cortex. We chose these VOIs as SERT binding in these regions is relevant in relation to previously described allopregnanolone actions (Arelin et al., 2015; Syan et al., 2017). Time-activity curves from the VOIs and cerebellum (nonspecific binding) were only obtained from gray matter voxels, except in the midbrain, where separation of gray and white matter is difficult.

Mood, and Alertness

Emotional functioning and mood symptoms were assessed using the following questionnaires: Profile of Mood States (POMS); Cohen's Perceived Stress Scale (PSS); Symptom Checklist-revised (SCL); and Global Severity Index of SLC score (SCL-GSI). A Simple Reaction Time task was performed to measure general alertness and motor speed, as described in (Stenbaek et al., 2016).

5-HTTLPR

The serotonin transporter-linked polymorphic region (5-HTTLPR) (Heils et al., 1996; Lesch et al., 1996) was genotyped in 87 of the participating women, as described in (Kalbitzer et al., 2010). Genotype frequencies were in Hardy-Weinberg equilibrium; SS = 22.2%, SL = 41.1%, and LL = 33.3%).

Statistics

The association between allopregnanolone serum concentrations and SERT binding was evaluated by multiple linear regression models. Previous findings have indicated that cerebral SERT binding is influenced by season, age (declining with increasing age), and body mass index (BMI) (Kalbitzer et al., 2010; Tyrer et al., 2016). For these reasons, the weighted least square regression models of SERT binding included age, BMI, and daylight minutes as covariates, all entered as continuous variables. Because allopregnanolone levels were not normally distributed, we also weighted the regression models against the rank order of allopregnanolone. Further all models were carefully checked to ensure that unstandardized residuals were normally distributed. Finally, we validated the weighted least square regression models by use of robust linear regression using an MM estimator.

Path analysis, with bootstrapping to yield confidence intervals for the indirect effect, was performed to evaluate if the effect of allopregnanolone on simple reaction latency was mediated by prefrontal cortex SERT binding. Statistical analyses were performed by SPSS 24.0, SPSS Amos 24.0, and R 3.4.3 using the RobustBase 0.92 package.

RESULTS

As described in **Table 1**, women were on average young adults, slightly over-weight and healthy from a psychiatric perspective. According to normative allopregnanolone levels (Nyberg et al., 2007), the majority of women was assessed in the follicular phase,

TABLE 1 | Demographic data, allopregnanolone levels, and [^{11}C]DASB binding in the brain.

	n = 90	
	Mean ± SD	**Range**
Age, years	27.4 ± 8.2	18–49
BMI, kg/m^2	25.4 ± 6.3	17.0–43.9
Allopregnanolone, nmol/l	0.61 ± 0.50	0.13–3.70
Estradiol, pmol/l[a]	203 ± 130	60–810
Progesterone, nmol/l[a]	0.2 ± 0.13	0.06–0.8
Daylight, minutes	739 ± 201	426–1052
SCL global severity index	0.3 ± 0.2	0.01–1.16
Total Mood Disturbance score	5.7 ± 20.5	−21–86
[^{11}C]DASB BP$_{ND}$		
Prefrontal cortex	0.38 ± 0.09	0.12–0.61
Midbrain	2.06 ± 0.29	1.22–2.71
Pallidostriatum	1.98 ± 0.35	1.12–3.02
Total amygdala	1.78 ± 0.35	0.82–3.04
Total insula	0.89 ± 0.14	0.42–1.32
Total hippocampus	0.71 ± 0.13	0.33–1.09
Total posterior cingulate	0.49 ± 0.10	0.16–0.69
Injected dose, MBq	571 ± 56	360–642
Injected mass per kilo, μg/kg	0.03 ± 0.04	0.00–0.24
Specific activity, GBq/mmol	177 ± 136	10.0–675

[a]*Estradiol and progesterone serum concentrations only available in 72 women. SCL-GSI, Global Severity Index of the Symptom Checklist-revised score.*

TABLE 2 | Multiple linear regression analyses on allopregnanolone influence on [^{11}C]DASB binding.

	Slope estimate	**SD**	**β**	p_{adj}	**Adjusted R^2**
Prefrontal cortex	−0.038	0.017	−0.248	0.024	0.06
Midbrain	−0.070	0.048	−0.149	0.147	0.16
Pallidostriatum	−0.172	0.006	−0.302	0.005	0.10
Total amygdala	−0.116	0.062	−0.146	0.067	0.06
Total insula	−0.072	0.025	−0.300	0.005	0.11
Total hippocampus	−0.062	0.022	−0.293	0.006	0.13
Total posterior cingulate	−0.044	0.016	−0.264	0.008	0.23

Models adjusted for age, BMI, daylight minutes on the scanning day, and weighted against rank order of allopregnanolone levels.

82/90 (91.1%). The highest SERT binding levels on average were found in the midbrain and pallidostriatum, whereas the lowest levels were noted in the prefrontal cortex and posterior cingulate (**Table 1**).

Serum allopregnanolone levels were negatively associated with SERT binding in the prefrontal cortex, pallidostriatum, insula, posterior cingulate, and hippocampus (**Table 2** and **Figure 1**). Overall, the models, which also included age, BMI and daylight minutes, explained 6–23% of the variance in SERT binding levels across regions of interest, with the highest explanatory value in the midbrain, and the lowest in the prefrontal cortex (prefrontal cortex: (slope = −0.038, *SD* = 0.017, β = −0.248, *p* = 0.024)

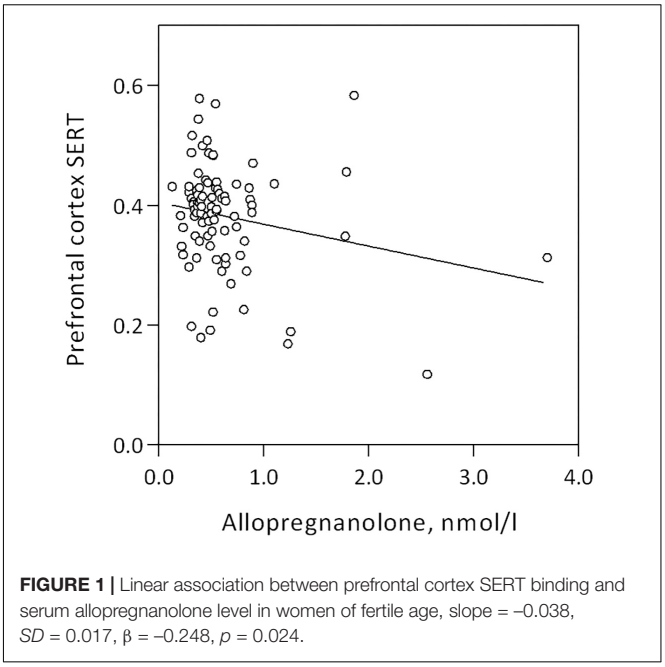

FIGURE 1 | Linear association between prefrontal cortex SERT binding and serum allopregnanolone level in women of fertile age, slope = –0.038, *SD* = 0.017, β = –0.248, *p* = 0.024.

TABLE 3 | Correlations between allopregnanolone and mood proxies.

	n	**Spearman Rho**	***p***
POMS Total Mood Disturbance (TMD) score	75	0.176	0.132
SCL-GSI	90	0.136	0.202
Cohen PSS	89	0.015	0.891
SRT total mean reaction latency	**64**	−0.267	**0.033**
SRT percent correct trials	**64**	**−0.309**	**0.013**

POMS, Profile of Mood States; PSS, Perceived Stress Scale; SCL, Symptom Checklist-revised; SCL-GSI, Global Severity Index of SLC score; SRT, Simple Reaction Test; TMD, Total Mood Disturbance. SRT, Simple Reaction Task.

(**Table 2**). However, the only allopregnanolone association that survived robust regression was the one with SERT binding in the prefrontal cortex (slope = −0.011, *SD* = 0.003, β = −0.196, $p < 0.001$), whereas the robust regressions between allopregnanolone and SERT binding in the pallidostriatum and hippocampus were in borderline significance (pallidostriatum; slope = −0.162, *SD* = 0.083, β = −0.236, *p* = 0.054, hippocampus; slope = −0.049, *SD* = 0.027, β = −0.187, *p* = 0.071). Further, the negative association between allopregnanolone and prefrontal cortex SERT binding remained when also adjusted for estradiol levels (slope = −0.092, *SD* = 0.029, β = −0.359, *p* = 0.002, *n* = 70). Considering 5-HTTLPR genotype, as a proxy of SERT functionality, did not influence any of the above mentioned findings (tested both as first order effects and interactions).

Allopregnanolone levels were negatively correlated with mean reaction latency and percent correct trials in the Simple Reaction Test, **Table 3** and **Figure 2**. Women who reacted slower displayed higher allopregnanolone levels, and similarly, made more errors (**Table 3**). The path analysis revealed that allopregnanolone exerted a significant direct effect on frontal cortex SERT binding (standardized estimate −0.321, *p* = 0.034) and a

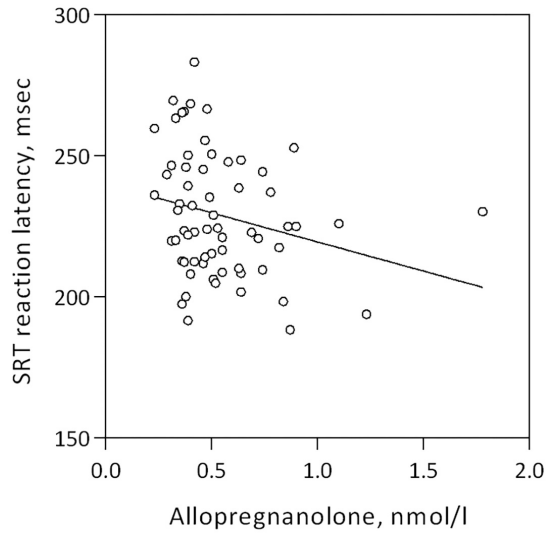

FIGURE 2 | Linear association between mean reaction latency in the simple reaction test and allopregnanolone levels in women of fertile age, Spearman's rho = –0.267, $p = 0.033$.

borderline significant effect on reaction latency (standardized estimate −0.179, $p = 0.061$). However, the indirect effect of allopregnanolone on reaction latency, mediated by prefrontal cortex SERT binding in the path model, was insignificant (standardized estimate −0.066, $p = 0.061$). Allopregnanolone serum concentrations did not influence any of the emotion- or stress-related measures (**Table 3**).

DISCUSSION

Mapping of the allopregnanolone-serotonin transporter relationship in women of fertile age demonstrated a negative correlation between peripheral levels of this neuroactive steroid and prefrontal cortex availability of the protein responsible for serotonin re-uptake from the synaptic cleft. The major limitations of this study was that measurements were mostly performed in the follicular phase, and that the study only included healthy women.

Allopregnanolone, produced both by the ovaries and in the brain, acts not only as a transcription regulator but also as a GABA agonist by binding to its site on the GABA receptor A ($GABA_A$). Allopregnanolone binding to the $GABA_A$ receptor leads to enhanced chloride ion flow and decreased GABA unbinding, which is reflected by increased frequency of inhibitory postsynaptic current and desensitization of the receptor (Wang, 2011). With GABA being the major inhibitory neurotransmitter in the brain, present in one third of all synapses, the effects of allopregnanolone are expected to be widely spread, albeit with some differences, depending on the localization and subunits of the $GABA_A$ receptor (Wang, 2011). As the women in this study were assessed at a time-point when progesterone and allopregnanolone levels are low, the present findings may reflect interactions between SERT and GABA-mediated tonic conductance at the extra-synaptic level, via $\alpha_4\beta_2\delta$ GABA receptors (Wang, 2011). Presence of $GABA_A$ receptors outside the synaptic cleft has been shown in the hippocampal formation and cortex (Wang, 2011), brain regions expressing SERT. Additionally, phasic modulation of neuronal excitability by GABA can be also influenced by synaptic $GABA_A$ receptors found ubiquitously in the brain (Wang, 2011). Clearly, longitudinal investigations will be needed to understand the relationship between serotonergic neurotransmission and fluctuations of allopregnanolone and mood across the menstrual cycle.

The present findings highlight a relationship between allopregnanolone and SERT, particularly in the prefrontal cortex. In this key region for higher cognitive functions and top-down regulation of emotions, GABA as well as serotonergic projections are widespread (Mengod et al., 2015). Some evidence on GABA-serotonin reciprocal modulation may be derived from animal studies (Svensson et al., 2000; Yan, 2002), but human data is scarce. In terms of molecular imaging of markers of serotonergic neurotransmission in relation to progesterone, $5\text{-}HT_{1A}$ receptor binding was negatively correlated with progesterone at the whole brain level in postmenopausal women (Stein et al., 2014), whereas combined estrogen-progesterone treatment in postmenopausal women was associated with widespread increased cortical $5\text{-}HT_{2A}$ receptor binding (Moses et al., 2000; Moses-Kolko et al., 2003). However, binding of these two receptors did not correlate with progesterone in another study of pre- and post-menopausal women (Moses-Kolko et al., 2011). To our knowledge, this is the first study investigating SERT binding in relation to the progesterone metabolite allopregnanolone in women of fertile age.

Higher SERT in the presence of low allopregnanolone levels, and independently of estradiol levels, may explain psychological well-being during the follicular phase of the menstrual cycle. In fact, no association of allopregnanolone with mood and stress was found in this group of healthy women, likely because of the healthy state and the limited variation in these measures during the follicular phase. Previously, lower SERT binding has been associated with depression (Parsey et al., 2006; Newberg et al., 2012). Dysregulation of both allopregnanolone and serotonin have been implicated in anxiety, irritability aggressiveness, as well as cognitive impairment (Schule et al., 2014); however, knowledge of their interaction at the molecular level is scarce. While enhancing effects of SSRIs on peripheral allopregnanolone levels have been demonstrated (Uzunov et al., 1996; Griffin and Mellon, 1999; Pinna et al., 2009), effects in the other direction remain to be studied. Additionally, neurotrophic-like functions have been demonstrated for both allopregnanolone and serotonin, though more limited to developmental stages for serotonin (Whitaker-Azmitia, 2001); and brain-derived neurotrophic factor (BDNF) may be one link. The less functional variant of a polymorphism in the brain derived-neurotrophic factor gene (*BDNF*), but not the short allele of 5-HTTLPR, has for example been associated with decreased fronto-cingulate activity in response to emotional stimuli in PMDD patients in the luteal phase

(Comasco et al., 2014). In line, no 5-HTTLPR genotype effect was observed in the present study.

We found a negative association between allopregnanolone and general alertness, as measured by the Simple Reaction Test. This finding was expected, given the agonistic effects of allopregnanolone on the $GABA_A$ receptor (Wang, 2011). In line with a direct effect on the $GABA_A$ receptor, we found no evidence that slower reaction times was mediated by lower SERT binding.

CONCLUSION

To conclude, considering strengths and limitations of the study, the present findings add knowledge to our understanding of allopregnanolone-SERT neurochemistry in brain regions regulating cognition and emotion processing of potential relevance for sex-specific psychiatric disorders. Further studies with samples from the luteal phase, and also including women with diagnosed PMDD, are needed before a full interpretation of the allopregnanolone-SERT relationship can be made.

AUTHOR CONTRIBUTIONS

IS, EC, and VF were involved in the conception and design of the study. TB, MB, and PJ participated in the acquisition of data. IS and EC did the data analyses. VF, TB, MB, and PJ contributed to the interpretation. IS and EC drafted the manuscript. All authors revised the manuscript critically for important intellectual content and approved the final version of the manuscript.

FUNDING

The funding body had no role in the design of the study, collection and analysis of data and decision to publish. IS and EC are supported by the Swedish Research Council. EC was a Marie Skłodowska Curie fellow and received funds from the Swedish Research Council (VR: 2015-00495), EU FP7-People-Cofund (INCA 600398), and SciLifeLab. VF was supported by The Danish Council for Independent Research, The Lundbeck Foundation (Cimbi), and The Capital Region of Denmark, Foundation for Health Research.

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Conflict of Interest Statement: IS serve occasionally on advisory boards or act as invited speaker at scientific meetings for MSD, Bayer Health Care, and Lundbeck A/S. VF has received honorarium as speaker for H Lundbeck A/S.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 23 November 2018
doi: 10.3389/fendo.2018.00703

A Novel, Synthetic, Neuroactive Steroid Is Effective at Decreasing Depression-Like Behaviors and Improving Maternal Care in Preclinical Models of Postpartum Depression

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OPEN ACCESS

Edited by:
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Reviewed by:
Benedetta Leuner, The Ohio State University, United States
James A. Carr, Texas Tech University, United States

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Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *10 July 2018*
Accepted: *08 November 2018*
Published: *23 November 2018*

Citation:
Melón L, Hammond R, Lewis M and Maguire J (2018) A Novel, Synthetic, Neuroactive Steroid Is Effective at Decreasing Depression-Like Behaviors and Improving Maternal Care in Preclinical Models of Postpartum Depression. Front. Endocrinol. 9:703. doi: 10.3389/fendo.2018.00703

Preclinical testing of treatments for postpartum depression (PPD) has been limited due to the lack of available animal models of such a complex disorder. To address this limitation, our laboratory has generated unique preclinical mouse models that exhibit abnormal postpartum behaviors. Mice with a loss or reduction in the expression of the $GABA_A$ receptor ($GABA_AR$) δ subunit (*Gabrd*$^{-/-}$ or *Gabrd*$^{+/-}$, respectively) and mice that lack the K^+/Cl^- co-transporter, KCC2, specifically in corticotropin-releasing hormone (CRH) neurons (KCC2/Crh mice) exhibit depression-like behaviors restricted to the postpartum period and deficits in maternal care, which serve as useful tools for testing novel therapeutic compounds. Utilizing these preclinical models, we tested the ability of a novel, synthetic, neuroactive steroid developed by SAGE Therapeutics, SGE-516, to improve abnormal postpartum behaviors. *Gabrd*$^{-/-}$, *Gabrd*$^{+/-}$, and KCC2/Crh dams treated with SGE-516 (450 mg/kg chow) during late pregnancy exhibit a decrease in depression-like behaviors and improvements in maternal care at 48 h postpartum. Interestingly, acute treatment with SGE-516 also exhibits robust therapeutic effects in these preclinical PPD models. We previously discovered abnormal stress reactivity associated with hypothalamic-pituitary-adrenal (HPA) axis dysregulation associated with depression-like behaviors in the preclinical PPD models, evident from an increase in stress-induced corticosterone levels and dephosphorylation and downregulation of KCC2 in the paraventricular nucleus of the hypothalamus (PVN) during the peripartum period. Here we demonstrated that SGE-516 treatment is sufficient to prevent the stress-induced increase in corticosterone and dephosphorylation and downregulation of KCC2 in the PVN. In contrast, and consistent with the distinct pharmacology of SGE-516 compared to benzodiazepines, treatment with clobazam (250 mg/kg chow) did not alter the depression-like phenotype or deficits in maternal care observed in these preclinical models of PPD. These findings are consistent with the positive double-blind, randomized, placebo-controlled trial findings of a similar compound, brexanolone, in the

treatment of patients with postpartum depression. Further, these findings validate the use of these preclinical models of PPD for screening novel compounds for the treatment of postpartum depression.

Keywords: neurosteroids, postpartum depression (PPD), HPA axis, benzodiazepine (BDZ), KCC2 = potassium chloride cotransporter 2

INTRODUCTION

Postpartum depression impacts nearly 20% of mothers (1, 2) and a much larger percentage (up to 75%) suffer from postpartum blues (3). Despite the high incidence of postpartum mood disorders, there has been a lack of research into the underlying biological mechanisms and potential treatments, in part, due to the lack of animal models required for preclinical research. Due to the time course of symptom presentation, the decline in ovarian hormones have been implicated in postpartum depression (4). However, women with postpartum depression do not exhibit differences in steroid hormone levels compared to controls [for review see (5)]. Interestingly, hormone withdrawal only induces depression symptoms in women with a history of postpartum depression (6), suggesting that they may be differentially sensitive to ovarian hormones. Women with postpartum depression do exhibit lower levels of allopregnanolone levels compared to healthy controls (7, 8), implicating neurosteroids and their site of action in postpartum depression. $GABA_AR$s are a principal target for neurosteroid action (9–12) [for review see (13, 14)], in particular extrasynaptic, $GABA_AR$ δ subunit containing receptors, which mediate tonic GABAergic inhibition and confer neurosteroid sensitivity (15). These receptors are unique from synaptic receptors, particularly those incorporating the $GABA_AR$ γ2 subunit, which are sensitive to benzodiazepines (15).

Our research previously demonstrated that mice that lack the $GABA_AR$ δ subunit, *Gabrd*$^{-/-}$ mice, exhibit abnormal postpartum behaviors, including depression-like behaviors restricted to the postpartum period and deficits in maternal care (10). It was previously demonstrated that the abnormal maternal care is due to deficits in the dam, not the pup, through cross-fostering experiments (10). Utilizing this model, our laboratory was able to investigate potential mechanisms contributing to the abnormal postpartum phenotype in this model, which pointed to dysregulation of the physiological response to stress, mediated by the HPA axis, during the postpartum period (16).

Based on these findings, we explored the regulation of the HPA axis during the peripartum period and identified a role for the K^+/Cl^- co-transporter, KCC2, which is required for effective GABAergic inhibition [for review see (17)], in the regulation of CRH neurons that control the body's physiological response to stress. The stress-induced activation of the HPA axis has been shown to involve compromised chloride homeostasis due to a dephosphorylation of KCC2 at residue Ser940 and downregulation of KCC2 in the PVN (18, 19). We previously demonstrated that the suppression of the HPA axis during the peripartum period involves maintenance of KCC2 expression in the PVN (20). To further examine the role of the HPA axis in contributing to vulnerability to mood disorders during the postpartum period, we generated mice that lack KCC2 specifically in CRH neurons (KCC2/Crh mice) (20). KCC2/Crh mice also exhibit depression-like behaviors and deficits in maternal care restricted to the postpartum period (20).

Here we utilize these preclinical PPD models to test the therapeutic effectiveness of GABA receptor modulators in the treatment of postpartum depression-like behaviors, specifically SGE-516, which is a novel, synthetic allopregnanolone analog, or clobazam, a benzodiazepine. Our data demonstrate that SGE-516 is effective in decreasing depression-like behaviors and improving maternal care in these preclinical PPD models. In contrast, the benzodiazepine, clobazam, is ineffective at altering abnormal postpartum behaviors in these preclinical PPD models. These results support a recent double-blind, randomized, placebo-controlled trial, demonstrating that brexanolone IV, a proprietary formulation of allopregnanolone, is effective in treating postpartum depression (21, 22) and validate the use of these preclinical models for assessing the effectiveness of potential treatments for PPD.

MATERIALS AND METHODS

Animals

Adult (>P60) female Wild Type (WT), *Gabrd*$^{-/-}$, *Gabrd*$^{+/--}$, or KCC2/Crh mice were bred and housed at Tufts University School of Medicine's Division of Laboratory Animal Medicine facility under a 14/10 light schedule (lights on at 7:00 h) with *ad libitum* access to food and water. Littermates were used for all behavioral experiments. Females were harem bred with a male and the presence of a vaginal plug was used to time pregnancy (Positive plug = day 0). The estrous cycle was not monitored in virgin animals since we have determined that females are acyclic under our housing conditions, which entails using ventilated racks and isolation by sex (20). This is not abnormal or stress related given that is well-established that female mice become acyclic (anestrus) without close proximity to a male or exposure to pheromone in his urine (23, 24). Experiments were performed in virgin and postpartum (48–72 h) females. All procedures were approved by the Tufts University Institutional Animal Care and Use Committee and adhered to the ethical guidelines presented in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (25).

Mice with a global knockout of the gene encoding the $GABA_AR$ δ subunit, *Gabrd*, (*Gabrd*$^{-/-}$ mice) were originally obtained from Dr. Istvan Mody (University of California at Los Angeles, UCLA). The abnormal postpartum phenotype in this model was characterized in a previous manuscript (10). Mice lacking KCC2 specifically in CRH neurons were

generated by crossing a floxed KCC2 ($KCC2^{f/f}$) mouse line (a generous gift from Dr. Stephen J. Moss) with CRH-Cre mice originally obtained from the Mutant Mouse Regional Research Center (Stock # 030850-UCD). This mouse model, including the postpartum depression-like phenotype, has been thoroughly characterized in a recent manuscript (20). The KCC2/Crh mice are maintained on a 129/Sv background; whereas, the $Gabrd^{-/-}$ mice are maintained on a C57Bl6/J background. Thus, these experiments assess the therapeutic effectiveness of compounds in ameliorating abnormal postpartum behavior in two different strains of mice.

Drug Treatments

Two different treatment strategies were employed in the current study, chronic administration of compounds that extends through late pregnancy and the early postpartum period and acute treatment exclusively during the postpartum period. Thus, the timing of these treatments will enable us to determine if treatment exclusively during the postpartum period is effective or if extended treatment is required. Chronic drug treatments were administered starting at day 14 of pregnancy and continuing until the end of experimentation at 48 h postpartum, lasting ∼6–9 d (∼7 d), the variability depending upon the length of gestation that ranged from 18 to 21 d). At day 14 of pregnancy, mice were randomly assigned to a treatment group and provided with standard chow or chow containing SGE-516 (450 mg/kg chow) or clobazam (250 mg/kg chow) until the time of testing. These doses were chosen because they result in plasma concentrations previously shown to exert centrally-acting effects (26).

For the acute drug treatments, mice were administered either vehicle (5% 2-hydroxypropyl-beta-cyclodextrin [HPβCD]), SGE-516 (5 mg/kg), or clobazam (10 mg/kg). The vehicle used (5% HPβCD) has previously been demonstrated to be inert (27). All drugs were administered by intraperitoneal (*i.p.*) injection 30 min prior to experimentation.

Pharmacokinetic studies were performed to determine the plasma and brain levels reached by both the acute and chronic treatment paradigms. Plasma samples were collected in K2EGTA-coated tubes and centrifuged at 2,000 G for 10 min at 4°C. Whole brains were weighed and snap frozen on dry ice. Plasma and brain samples were stored at−80°C until use. Exposure levels in the tissue and plasma were determined by Pharmacadence (Hatfield, PA). The plasma and brain exposure levels are provided in **Table 1**. The amount of chow consumed (standard chow: 6.7 ± 0.9 g/day; SGE-516 chow: 6.0 ±0.4 g/day) was not different between treatment groups (data not shown). No signs of sedation or overt changes in health were observed in any of the treatment groups. Homecage locomotor activity was not altered in SGE-516 treated dams (2167.3 ± 75.8 cm/day) compared to standard chow treatment (2351.6 ± 346.6 cm/day). The higher exposure levels following the acute SGE-516 treatment is likely due to the likely due to the route of administration. In the chronic SGE-516 treatment, the mice consume the chow largely during the dark period with levels peaking during this phase. The tissue and plasma were collected in the morning and may not reflect peak exposure values in the chronic SGE-516-treated group; whereas, the acute SGE-516 treatment was performed 30 min prior to sample collection, likely contributing to the higher levels observed following the acute treatment.

To assess whether SGE-516 can alter KCC2 expression in the PVN using Western blot analysis, we employed virgin adult female mice (60–90 d of age) to prevent normal peripartum hormonal changes from confounding the results. Virgin females were maintained on either standard chow or SGE-516 chow (450 mg/kg chow) for 18 consecutive days to mimic the elevated levels of neurosteroids throughout pregnancy.

Behavioral Tests

Behavioral tests were conducted on postpartum WT, $Gabrd^{-/-}$, $Gabrd^{\pm/--}$, and KCC2/Crh mice maintained on standard, SGE-516, or clobazam chow or acutely administered vehicle, SGE-516, or clobazam 30 min prior to testing. Animals were subjected to both the forced swim test and the maternal approach test. All behavioral tests occurred between 09:00 and 16:00 h, following at least 1 h of habituation in the behavioral testing room. The behavioral tests were videotaped and scored by two different investigators, one blinded to the experimental condition. This approach was utilized to prevent subjective interpretation of the data. There was no significant difference in the results of the independent scoring; therefore, the analysis from the blinded investigator was used for the final data sets.

The forced swim was conducted as previously described (10, 16, 20). Mice were individually placed into a plastic beaker (21 cm diameter) containing 15 cm of room temperature water (23–25°C). The latency to immobility and the total time spent immobile was measured over the 6 min test. Immobility was considered to be floating with no front paw movement and no movement or minimal movement of a single hind paw. In contrast, directional swimming and active struggling were not included in the time spent immobile. Following the forced swim test, mice were towel dried and placed back into their homecage.

The maternal approach test was performed following the forced swim test as previously described to investigate the impact of stress during the postpartum period on maternal behavior (20). The dams were introduced into their homecage at the opposite corner from their litter after a brief separation (10 min). The latency to approach the pups and the total time spent in contact with pups were measured as indices of maternal behavior. Only >5 s interactions were included in the time spent with the pups. Burrowing beneath or trampling the litter was not included in the measure of pup contact time.

Corticosterone Measurements

Peripartum HPA axis abnormalities were assessed by subjecting virgin mice and postpartum mice to an acute restraint stress (30 min) by placing mice individually into a 50 mL Falcon tube modified with breathing holes and comparing corticosterone levels to unstressed homecage controls. Mice subjected to the 30 min restraint stress or minimally handled controls were anesthetized with isoflurane, rapidly decapitated, trunk blood was collected in CAPIJECT® (T-MG) tubes, and serum collected by centrifugation for corticosterone measurements. All samples were collected between 10:00 and 12:00 h. Corticosterone

TABLE 1 | Pharmacological treatments and exposure levies.

Experiment	Dose	Route of administration	Plasma levels (ng/ml)	Brain levels (ng/g)	n numbers
Vehicle	standard chow	oral (chow)	nd	nd	2
Chronic SGE-516	450 mg/kg chow	oral (chow)	72.5 ± 17.4	86.6 ± 211	4
Chronic clobazam	250 mg/kg chow	oral (chow)	54.3 ± 9.9	73.1 ± 11.9	9
Vehicle	5%HPβCD	i.p.	nd	nd	2
Acute SGE-516	5mg/kg	i.p.	415.0 ± 174.8	669.8 ± 265.4	4
Arute Clobazam	10mg/kg	i.p.	94.1 ± 18.6	180.6 ± 27.9	5

i.p., Intraperitoneally; nd, not detected.

was measured as previously described (20, 28, 29), using a commercially available enzyme immunoassay kit, according to the manufacturer's instructions (Enzo Pharmaceuticals, New Jersey). Briefly, samples were run in duplicate and compared to a standard curve of known corticosterone concentrations.

Western Blot

Western blots were carried out as previously described (19, 20, 29, 30). Mice were anesthetized between 10:00 and 12:00 h with isoflurane, sacrificed by guillotine-assisted decapitation, and the PVN was microdissected and placed in ice-cold homogenization buffer (10 mM NaPO4, 100 mM NaCl, 10 mM Na pyrophosphate, 25 mM NaF, 5 mM EDTA, 5 mM EGTA, 2% Triton X-100, 0.5% Deoxycholate, 1 mM Na vanadate, pH 7.4), in the presence of protease inhibitors (complete mini, Roche, in fresh 100 mM PMSF dissolved in ethanol). Total protein was isolated and concentrations were determined using DC Protein Assay (Bio-Rad, Hercules, CA). Total protein (25 μg) was loaded onto a 12% SDS-polyacrylamide gel, subjected to electrophoresis and transferred to a PDVF membrane (Immobilon P, Millipore, Temecula, CA), blocked in 10% nonfat milk, and probed with a polyclonal antibody specific for KCC2 (1:1,000, Millipore, Temecula, CA), a phospho-specific antibody for phosphorylation of KCC2 at residue Ser940 (1:1,000, a generous gift from Dr. Stephen J. Moss), or a monoclonal β-tubulin antibody (1:10,000, Sigma Aldrich, St. Louis, MO). The blots were then incubated with either peroxidase labeled anti-rabbit IgG (1:2,500, GE Healthcare) or peroxidase labeled anti-mouse IgG (1:2,500, GE Healthcare) and immunoreactive proteins were visualized using enhanced chemiluminescence (Amersham/GE Healthcare). All experimental groups were run in parallel. Optical density measurements were performed using NIH ImageJ software and normalized to total protein levels (25 μg total protein) rather than a housekeeping protein, which have shown variability in expression levels (31).

Statistical Analyses

All data were analyzed using GraphPad Prism 6 or Excel. Statistical significance between vehicle and SGE-516 treatment groups for behavioral tests was determined using an unpaired Student's *t*-test. A Mann–Whitney non-parametric test was also performed to verify statistical significance. For all behavioral tests, ANOVAs were not performed to compare genotype and drug because the preclinical PPD models are maintained on different backgrounds and therefore direct comparisons are not appropriate. Further, the vehicles for the drugs are also different, requiring comparison to their respective vehicle controls. Thus, the comparison focused on the effect of drug vs. vehicle, in which a *t*-test is appropriate. Comparison between stress status (control and stress) and treatment (standard chow vs. SGE-516) for the Western blot experiments, on the other hand, was conducted using a two-way ANOVA with a Sidak *post-hoc* test for multiple comparisons. All data are represented as the average + SEM. Statistical significance was defined as $p < 0.05$.

RESULTS

Chronic SGE-516 Treatment Decreases Depression-Like Behaviors in Preclinical PPD Models

Gabrd$^{-/-}$, *Gabrd*$^{+/-}$, and KCC2/Crh mice exhibit depression-like behaviors during the postpartum period compared to their respective wild type controls (**Table 2**) (10, 16, 20). *Gabrd*$^{-/-}$, *Gabrd*$^{+/-}$, and KCC2/Crh mice exhibit a decrease in the latency to immobility and an increase in the total time spent immobile in the forced swim test compared to wild type at 48 h postpartum (**Table 2**; $p < 0.05$ using a Student's *t*-test compared to wild type). Here we demonstrate that chronic treatment of these preclinical PPD models with SGE-516 decreases depression-like behaviors during the postpartum period. *Gabrd*$^{-/-}$ dams treated with SGE-516 exhibit an increase in the latency to the first bout of immobility and a decrease in the total time immobile compared to standard chow-treated *Gabrd*$^{-/-}$ dams [**Figure 1A**, **Table 2**; $p < 0.05$ using a Student's *t*-test; latency: $t_{(17)} = -2.97$; total time immobile; $t_{(17)} = 6.59$]. Similarly, chronic treatment of *Gabrd*$^{+/-}$ dams with SGE-516 results in an increase the latency to immobility and a decrease in the total time immobile in the forced swim test compared to standard chow-treated *Gabrd*$^{+/-}$ dams [**Figure 1B**, **Table 2**; $p < 0.05$ using a Student's *t*-test; latency: $t_{(20)} = -2.01$; total time immobile; $t_{(20)} = 4.60$]. Mice lacking KCC2 in CRH neurons (KCC2/Crh) also exhibit depression-like behaviors restricted to the postpartum period that improves with SGE-516 treatment. SGE-516 treatment increases the latency to immobility and decreases the total time spent immobile in KCC2/Crh dams in the forced swim test compared to their standard chow-treated counterparts [**Figure 1C**, **Table 2**; $p < 0.05$ using a Student's *t*-test; latency: $t_{(25)} = -2.29$; total time

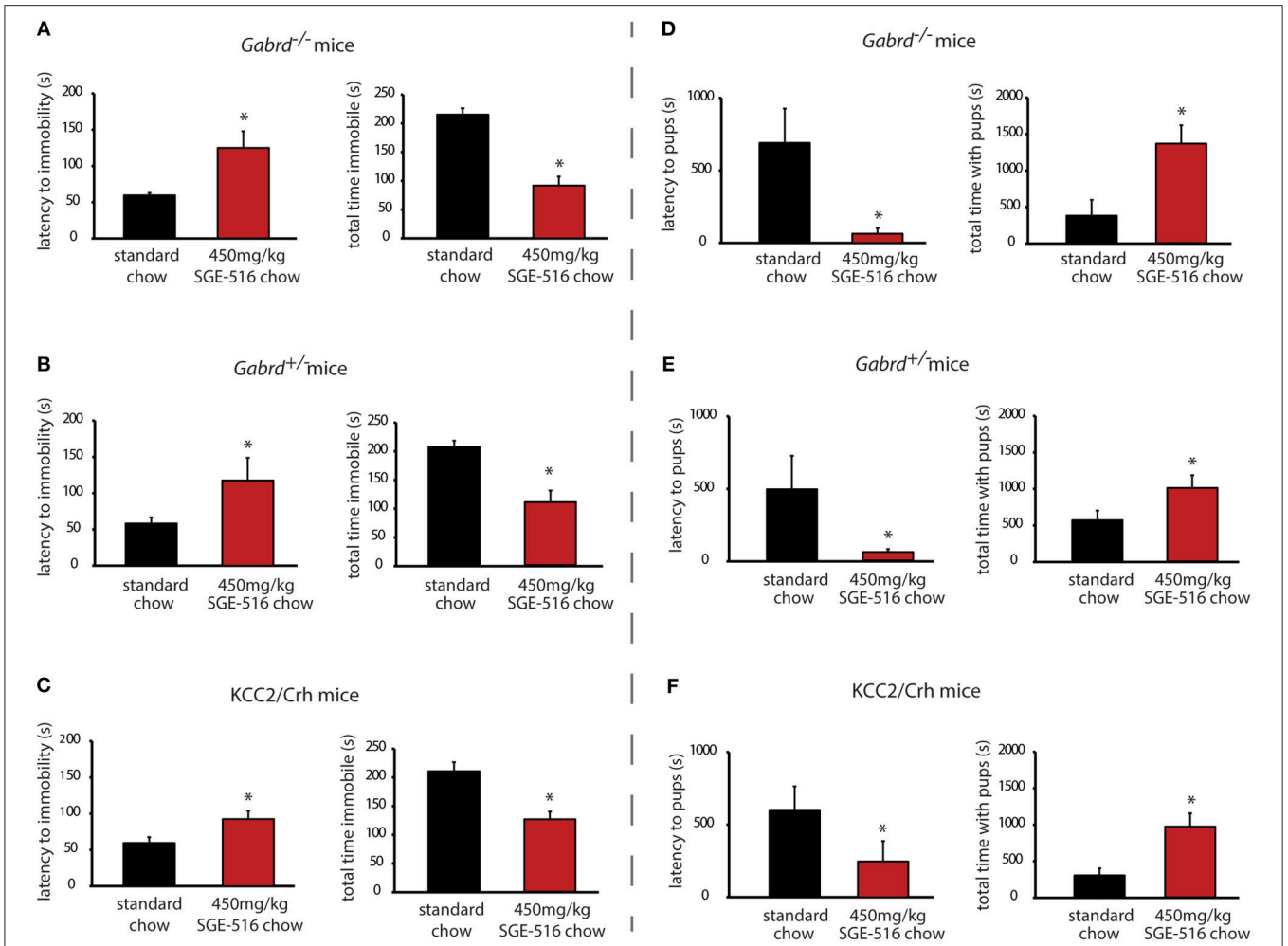

FIGURE 1 | Chronic SGE-516 treatment decreases depression-like behaviors and improves maternal care in preclinical PPD models**.** Chronic treatment with SGE-516 (450 mg/kg chow, ∼7 d) increases the average latency to immobility and decreases the total time spent immobile in postpartum *Gabrd*$^{-/-}$ **(A)**, *Gabrd*$^{+/-}$ **(B)**, and KCC2/Crh dams **(C)** in the forced swim test. *Denotes statistical significance of $p < 0.05$ using a Student's *t*-test. Chronic treatment with SGE-516 (450 mg/kg chow, ∼7 d) decreases the average latency to approach their pups and increases to total interaction time with the pups in postpartum *Gabrd*$^{-/-}$ **(D)**, *Gabrd*$^{+/-}$ **(E)**, and KCC2/Crh dams **(F)** in the maternal approach test. *Denotes statistical significance of $p < 0.05$ using a Student's *t*-test.

immobile; $t_{(25)} = 4.10$]. It is important to note that SGE-516 treatment does not alter behavior in the forced swim test in postpartum wild type mice (latency: 91.3 ± 3.8 s; total time immobile: 119.8 ± 14.2 s) compared to standard chow-treated wild type dams (latency: 82.2 ± 7.9 s; total time immobile: 131.3 ± 14.3 s) [data not shown; $p > 0.05$ using a Student's *t*-test; latency: $t_{(11)} = -0.73$; total time immobile; $t_{(11)} = 0.49$].

Chronic SGE-516 Treatment Improves Deficits in Maternal Care in Preclinical PPD Models

Gabrd$^{-/-}$, *Gabrd*$^{+/-}$, and KCC2/Crh mice exhibit deficits in maternal care compared to wild type dams (**Table 2**) (10, 20). *Gabrd*$^{-/-}$, *Gabrd*$^{+/-}$, and KCC2/Crh mice exhibit an increase in the latency to approach their pups and exhibit a decrease in the total time interacting with their pups in the maternal approach test compared to their respective wild types (**Table 2**; $p < 0.05$ using a Student's *t*-test compared to wild type). SGE-516 treatment improves maternal care in preclinical PPD models. Chronic treatment of *Gabrd*$^{-/-}$ mice with SGE-516 decreases the latency to approach and increases the total time interacting with their pups in the maternal approach test compared to standard chow-treated *Gabrd*$^{-/-}$ dams [**Figure 1D**, **Table 2**; $p < 0.05$ using a Student's *t*-test; latency to approach: $t_{(15)} = 2.67$; total interaction time; $t_{(15)} = -3.50$]. Similarly, treatment of *Gabrd*$^{+/-}$ dams with SGE-516 decreases the latency to approach and increases the total interaction time with their pups compared to standard chow-treated *Gabrd*$^{+/-}$ dams [**Figure 1E**, **Table 2**; $p < 0.05$ using a Student's *t*-test; latency to approach: $t_{(18)} = 2.07$; total interaction time; $t_{(18)} = -2.68$). Maternal care in KCC2/Crh dams is also improved with SGE-516 treatment,

TABLE 2 | Acute and Chronic SGE-516 treatment improves abnormal postpartum behaviors in two preclinical PPD models.

Genotype	Treatment	Latency to immobility	Total time immobile	*n*	Latency to approach	Total interaction time	*n*
Wild Type	Standard Chow	82.2 ± 7.9 s	131.3 ± 14.3 s	9	54.2 ± 12.6 s	1408.2 ± 187.2 s	8
Gabrd$^{-/-}$dams	Standard Chow	59.5 ± 3.2 s#	215.7 ± 11.1 s#	10	697.5 ± 197.3 s#	429.7 ± 177.8 s#	10
Gabrd$^{-/-}$dams	Chronic SGE-516 (450 mg/kg chow)	124.8 ± 23.0 s*	92.3 ± 15.4 s*	9	58.6 ± 33.0 s*	1411.9 ± 219.3 s	7
Gabrd$^{-/-}$dams	Standard Chow	54.3 ± 8.6 s#	227.6 ± 18.7 s#	7	752.9 ± 167.7 s#	376.4 ± 151.2 s#	12
Gabrd$^{-/-}$dams	Chronic Clobazam (250 mg/kg chow)	54.4 ± 6.9 s	236.9 ± 8.3 s	9	405.0 ± 143.6 s	133.6 ± 34.1 s	7
Gabrd$^{+/-}$dams	Standard Chow	57.5 ± 7.8 s#	207.1 ± 10.4 s#	12	580.5 ± 226.7 s#	529.5 ± 125.8 s#	11
Gabrd$^{+/-}$dams	Chronic SGE-516 (450 mg/kg chow)	112.0 ± 28.4 s*	113.5 ± 18.5 s*	10	57.8 ± 17.6 s*	1081.0 ± 168.4 s*	9
KCC2/Crh	Standard Chow	59.5 ± 8.1 s#	207.8 ± 15.3 s#	10	602.1 ± 162.5 s#	301.0 ± 96.4 s#	14
KCC2/Crh	Chronic SGE-516 (450 mg/kg chow)	95.9 ± 11.2 s*	125.7 ± 12.4 s*	17	229.2 ± 129.9 s*	973.9 ± 169.6 s*	13
KCC2/Crh	Standard Chow	54.5 ± 7.6 s#	217.8 ± 15.2 s#	10	655.5 ± 147.7 s#	310.9 ± 86.1 s#	11
KCC2/Crh	Chronic Clobazam (250 mg/kg chow)	40.0 ± 6.3 s	241.1 ± 7.2 s	8	801.4 ± 223.6 s	310.7 ± 127.6 s	7
Gabrd$^{-/-}$dams	Vehicle	62.8 ± 5.7 s	233.4 ± 18.2	9	901.4 ± 195.3 s	358.4 ± 141.3 s	11
Gabrd$^{-/-}$dams	Acute SGE-516 (5 mg/kg)	85.4 ± 4.9 s*	175.0 ± 15.7 s*	12	143.3 ± 96.2 s*	725.0 ± 166.8 s*	9
Gabrd$^{-/-}$dams	Vehicle	56.3 ± 8.1 s	218.5 ± 15.6 s	8	774.4 ± 207.1 s	317.1 ± 163.3 s	8
Gabrd $^{-/-}$dams	Acute Clobazam (10 mg/kg)	59.4 ± 5.0 s	253.4 ± 5.0 s	9	1143.5 ± 242.8 s	152.0 ± 73.8 s	10
KCC2/Crh	Vehicle	60.9 ± 6.9 s	221.2 ± 15.2 s	11	791.3 ± 191.2 s	232.3 ± 94.2 s	16
KCC2/Crh	Acute SGE-516 (5 mg/kg)	94.5 ± 9.5 s*	154.2 ± 17.0 s*	11	108.3 ± 66.1 s*	723.1 ± 129.6 s*	9
KCC2/Crh	Vehicle	61.9 ± 9.2 s	206.0 ± 18.3 s	8	884.3 ± 249.2 s	258.5 ± 139.3 s	10
KCC2/Crh	Acute Clobazam (10 mg/kg)	55.5 ± 9.3 s	252.7 ± 9.2 s	10	806.9 ± 206.1 s	398.8 ± 190.3 s	8

#*Denotes $p < 0.05$ compared to Wild Type mice treated with vehicle or standard chow; *Denotes $p < 0.05$ compared to vehicle or standard chow treated mice within genotype.*

with a decrease in the latency to approach their pups and a significant increase in the total interaction time compared to compared to standard chow-treated postpartum KCC2/Crh mice [**Figure 1F**, **Table 2**; $p < 0.05$ using a Student's *t*-test; latency to approach: $t_{(27)} = 1.73$; total interaction time; $t_{(27)} = -3.51$]. However, SGE-516 treatment does not alter maternal care measured using the maternal approach test in postpartum wild type mice (latency: 503.0 ± 286.3 s; total interaction time: 1292.0 ± 232.6 s) compared to standard chow-treated wild type dams (latency: 490.6 ± 285.9 s; total interaction time: 1056.1 ± 268.1 s) [data not shown; $p > 0.05$ using a Student's *t*-test; latency: $t_{(11)} = -0.03$; total time immobile; $t_{(11)} = -0.61$].

SGE-516 Suppresses the Stress-Induced Activation of the HPA Axis

To investigate whether the therapeutic effects of SGE-516 may involve regulation of the HPA axis, virgin wild type mice were treated with either standard chow or SGE-516 (18 d treatment) and baseline and stress-induced corticosterone levels were measured. Baseline corticosterone levels are not different between standard chow (25.0 ± 4.5 ng/ml) or SGE-516 (21.3 ± 2.6 ng/ml) treated mice. However, following a single, 30 min restraint stress, corticosterone levels are decreased in SGE-516-treated mice (74.2 ± 19.7 ng/ml) compared to standard chow-treated mice (303.2 ± 73.3 ng/ml) (**Figure 2A**; $n = 9$–11 mice per experimental group). There is a significant interaction between treatment and stress status in corticosterone levels [$p < 0.05$ using a two-way ANOVA; $F_{(1,36)} = 10.83$].

To examine whether SGE-516 suppresses the stress-induced activation of the HPA axis involving a KCC2-dependent mechanism, we examined KCC2 expression in the PVN in standard chow- and SGE-516-treated mice in unstressed, minimally handled controls and in mice subjected to a 30 min restraint stress. As previously demonstrated, acute restraint stress decreases the phosphorylation of KCC2 at residue Ser940 (34.5 ± 3.2 O.D. units/25 μg total protein) and decreases total KCC2 expression in the PVN (42.2 ± 5.6 O.D. units/25 μg total protein) compared to minimally handled controls (P-KCC2: 48.0 ± 4.1 O.D. units/25 μg total protein; KCC2: 60.4 ± 5.5 O.D. units/25 μg total protein) (**Figures 2B–D**). SGE-516 treatment prevents the stress-induced dephosphorylation of KCC2 at residue Ser940 (54.1 ± 4.1 O.D. units/25 μg total protein) and the downregulation of KCC2 expression in the PVN (73.5 ± 5.4 O.D. units/25 μg total protein) compared to minimally handled SGE-treated mice (P-KCC2: 46.6 ± 3.4 O.D. units/25 μg total protein; KCC2: 61.4 ± 1.9 O.D. units/25 μg total protein) and minimally handled, standard chow-treated controls (P-KCC2: 48.0 ± 4.1 O.D. units/25 μg total protein; KCC2: 60.4 ± 5.5 O.D. units/25 μg total protein) (**Figures 2B–D**). There is a significant interaction between treatment and stress status in the phosphorylation of KCC2 at residue Ser940 [$p < 0.05$ using a

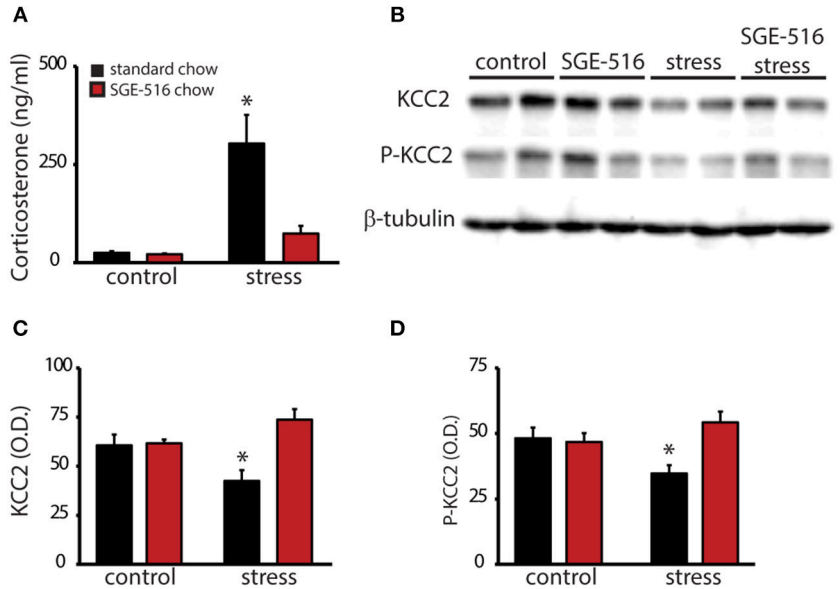

FIGURE 2 | SGE-516 mimics suppression of the HPA axis during the peripartum period. **(A)** The average baseline and stress-induced circulating corticosterone levels in virgin mice treated with standard or SGE-516 chow (450 mg/kg chow, 18 d). Stress-induced elevations in corticosterone are decreased in mice treated with SGE-516 compared to standard chow-treated mice. **(B)** Representative Western blots of total protein isolated from the PVN of virgin mice maintained on either standard or SGE-516 chow and subjected to a 30 min restraint stress or minimally-handled controls and probed with an antibody specific for KCC2, phosphorylated KCC2 at residue Ser940 (P-KCC2), or β-tubulin. The average optical density of KCC2 **(C)** and P-KCC2 **(D)** expression is decreased following stress in mice maintained on standard chow but not in mice maintained on SGE-516 chow. *Denotes statistical significance of $p < 0.05$ using a two-way ANOVA with a Sidak *post-hoc* test for multiple comparisons.

two-way ANOVA; $F_{(1,36)} = 7.796$] and total KCC2 expression [$p < 0.05$ using a two-way ANOVA; $F_{(1,36)} = 9.707$).

Chronic Clobazam Treatment Is Ineffective at Altering the Depression-Like Phenotype in Preclinical PPD Models

In contrast to SGE-516, the benzodiazepine, Clobazam, is ineffective at altering depression-like behaviors in postpartum *Gabrd*$^{-/-}$ or KCC2/Crh mice. Chronic clobazam treated *Gabrd*$^{-/-}$ mice exhibit a similar latency to immobility and total time spent immobile in the forced swim test compared to standard chow-treated *Gabrd*$^{-/-}$ dams [**Figure 3A**, **Table 2**; ns determined using a Student's *t*-test; latency: $t_{(14)} = -0.01$; total time immobile; $t_{(14)} = -0.49$].

Similarly, clobazam is ineffective at reducing depression-like behaviors in postpartum KCC2/Crh mice. The latency to immobility is similar between clobazam and standard chow-treated KCC2/Crh dams and the total time immobile is not significantly different between these treatment groups [**Figure 3B**, **Table 2**; ns determined using a Student's *t*-test; latency: $t_{(16)} = 1.42$; total time immobile; $t_{(16)} = -1.27$].

Chronic Clobazam Treatment Is Ineffective at Altering the Deficits in Maternal Care in Preclinical PPD Models

Unlike SGE-516, clobazam is also ineffective at improving maternal care in *Gabrd*$^{-/-}$ and KCC2/Crh dams. The latency to approach their pups in the maternal approach test is not affected by clobazam treatment compared to standard chow-treated controls [**Figure 3C**, **Table 2**; ns determined using a Student's *t*-test; *Gabrd*$^{-/-}$: $t_{(17)} = 1.41$; KCC2/Crh: $t_{(16)} = -0.57$]. The total interaction time in the maternal approach test is also not significantly different between clobazam-treated KCC2/Crh dams and standard chow-treated controls [**Figure 3D**, **Table 2**; ns determined using a Student's *t*-test; *Gabrd*$^{-/-}$: $t_{(17)} = 1.20$; KCC2/Crh: $t_{(16)} = 0.01$].

Acute SGE-516 Treatment Improves the Depression-Like Behaviors in Preclinical PPD Models

To further explore the time course of the therapeutic effects of SGE-516, we examined the ability of acute SGE-516 treatment to alleviate depression-like behaviors in preclinical PPD models. Acute SGE-516 treatment (5 mg/kg, *i.p.*) decreased depression-like behaviors during the postpartum period in both *Gabrd*$^{-/-}$ and KCC2/Crh mice. The latency to the first bout of immobility in the forced swim test is increased and the total time spent immobile is decreased in acute SGE-516-treated *Gabrd*$^{-/-}$ dams compared to vehicle treated mice [**Figure 4A**, **Table 2**; $p < 0.05$ using a Student's *I*-test; latency: $t_{(19)}$ =-3.03; total time immobile; $t_{(19)} = 2.43$]. Similarly, acute SGE-516 treatment in KCC2/Crh dams increases the latency to immobility and decreases the total time spent immobile in the forced swim test compared to vehicle-treated KCC2/Crh dams [**Figure 4B**, **Table 2**; $p < 0.05$ using a

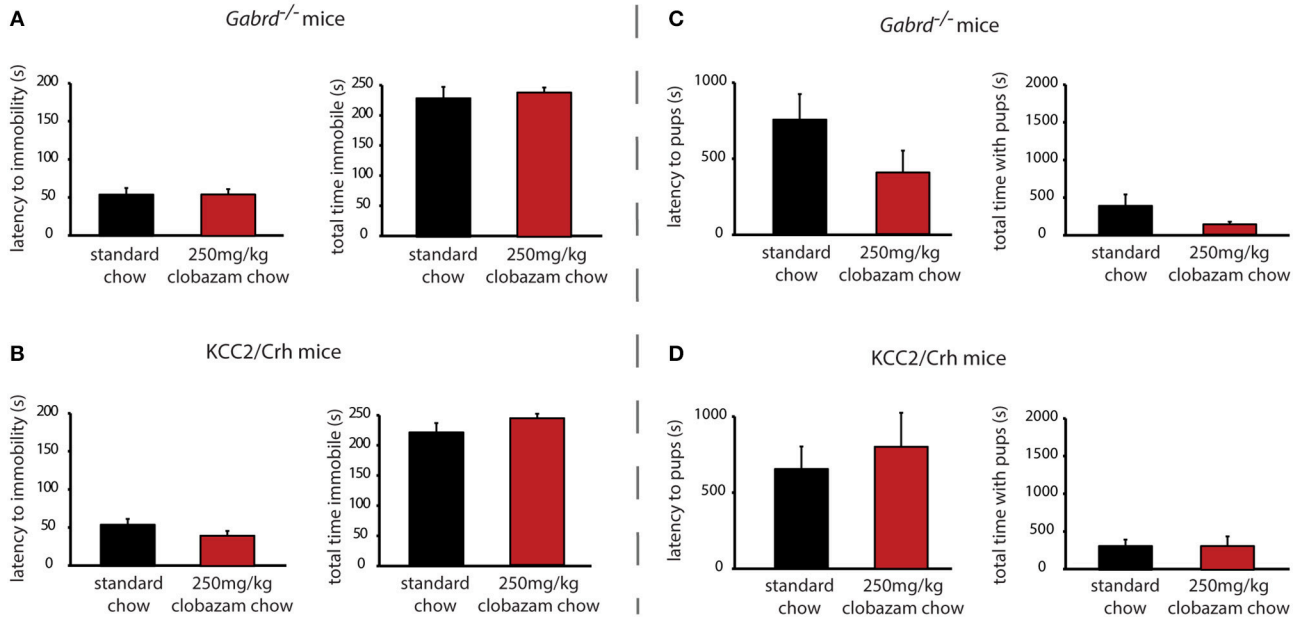

FIGURE 3 | Chronic clobazam is ineffective at altering depression-like behaviors or improving maternal care in preclinical PPD models. Treatment with clobazam (250 mg/kg chow, ∼7 d) does not alter the average latency to immobility or the total time spent immobile in postpartum *Gabrd*$^{-/-}$ **(A)** or KCC2/Crh dams **(B)** in the forced swim test. Treatment with clobazam (250 mg/kg chow, ∼7 d) does not alter the average latency to approach or the total pup interaction time in postpartum *Gabrd*$^{-/-}$ **(C)** or KCC2/Crh dams **(D)** in the maternal approach test.

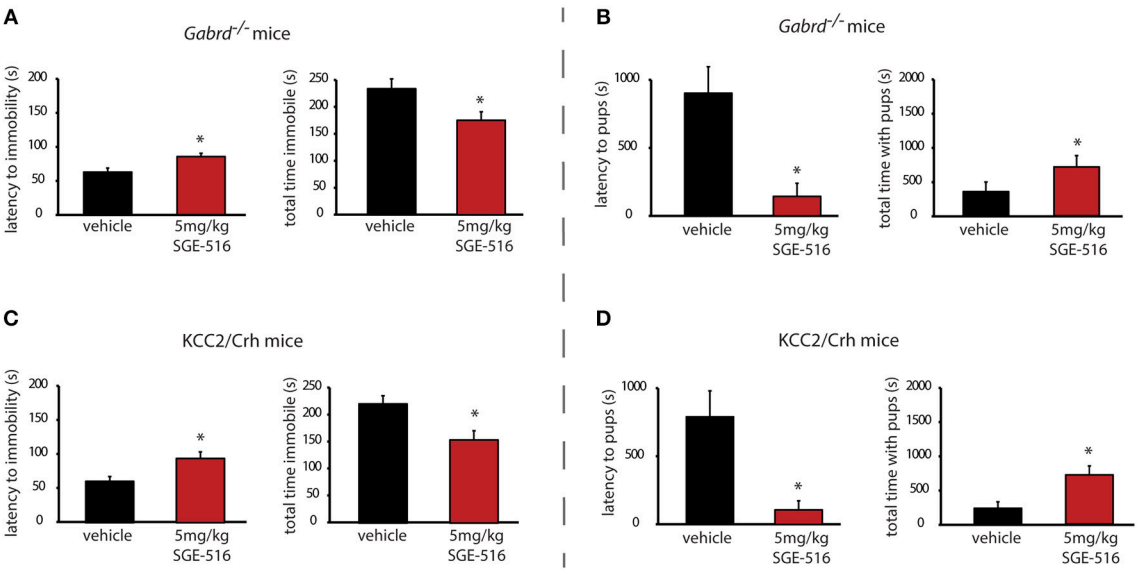

FIGURE 4 | Acute SGE-516 treatment decreases depression-like behaviors and improves maternal care in preclinical PPD models**.** Acute treatment with SGE-516 (5 mg/kg, 30 min prior to testing) increases the average latency to immobility and decreases the total time spent immobile in postpartum *Gabrd*$^{-/-}$ **(A)** and KCC2/Crh dams **(B)** in the forced swim test. *Denotes statistical significance of $p < 0.05$ using a Student's *t*-test. Acute treatment with SGE-516 (5 mg/kg, 30 min prior to testing) decreases the average latency to approach their pups and increases to total interaction time with the pups in postpartum *Gabrd*$^{-/-}$ **(C)** and KCC2/Crh dams **(D)** in the maternal approach test. *Denotes statistical significance of $p < 0.05$ using a Student's *t*-test.

Student's *t*-test; latency: $t_{(20)} = -2.86$; total time immobile; $t_{(20)} = 2.93$].

Acute SGE-516 Treatment Improves the Deficits in Maternal Care in Preclinical PPD Models

Acute SGE-516 treatment also improves maternal care in *Gabrd*$^{-/-}$ and KCC2/Crh dams. The latency to approach their pups is decreased and the total interaction time in the maternal approach test is increased in acute SGE-516 treated *Gabrd*$^{-/-}$ and KCC2/Crh dams compared to vehicle-treated controls [**Figures 4C,D**, **Table 2**; $p < 0.05$ using a Student's *t*-test; *Gabrd*$^{-/-}$: latency: $t_{(18)} = 3.25$; total interaction time; $t_{(18)} = -1.68$; KCC2/Crh: latency: $t_{(23)} = 2.61$; total interaction time; $t_{(23)} = -3.12$].

Acute Clobazam Treatment Does Not Alter the Depression-Like Phenotype in Gabrd$^{-/-}$ and KCC2/Crh Mice

Similar to the results obtained with chronic clobazam treatment, acute clobazam treatment is also ineffective at altering the depression-like phenotype observed during the postpartum period in preclinical PPD models. There is no difference in the latency to immobility in the forced swim test in acute clobazam-treated *Gabrd*$^{-/-}$ or KCC2/Crh dams compared to vehicle-treated controls [**Figures 5A,B**, **Table 2**; ns determined using a Student's *t*-test; *Gabrd*$^{-/-}$: $t_{(15)} = -0.34$; KCC2/Crh: $t_{(16)} = 0.48$]. Similarly, acute clobazam treatment does not alter the total time spent immobile in the forced swim test in *Gabrd*$^{-/-}$ or KCC2/Crh dams compared to vehicle-treated controls [**Figures 5A,B**, **Table 2**; ns determined using a Student's *t*-test; *Gabrd*$^{-/-}$: $t_{(15)} = -2.11$; KCC2/Crh: $t_{(16)} = -2.42$].

Acute Clobazam Treatment Does Not Alter the Deficits in Maternal Care in Gabrd$^{-/-}$ and KCC2/Crh Mice

Again, similar to the results obtained with chronic clobazam treatment, acute clobazam treatment does not alter the deficits in maternal care observed in preclinical PPD models. Acute clobazam treatment in *Gabrd*$^{-/-}$ dams does not alter the latency to approach or the total interaction time with their pups in the maternal approach test compared to vehicle-treated *Gabrd*$^{-/-}$ dams [**Figure 5C**, **Table 2**; ns determined using a Student's *t*-test; latency to approach: $t_{(16)} = 1.73$; total interaction time: $t_{(16)} = 0.99$]. Clobazam treatment in KCC2/Crh mice also does not change the latency to approach their pups or total pup interaction time in the maternal approach test compared to vehicle-treated KCC2/Crh dams [**Figure 5D**, **Table 2**; ns determined using a Student's *t*-test; latency to approach: $t_{(16)} = 0.11$; total interaction time: $t_{(16)} = -0.59$].

DISCUSSION

Here we demonstrate that U treatment with SGE-516 is effective at ameliorating abnormal postpartum behaviors in preclinical PPD models, decreasing depression-like behaviors and improving maternal care. The evidence that acute treatment with SGE-516 is effective in preclinical PPD models is translationally important, suggesting potential fast-acting treatment effects, which is consistent with the rapid onset of observed with a similar compound, brexanolone, in clinical trials (21, 22).

These therapeutic effects of SGE-516 in preclinical PPD models are distinct and do not translate to other GABAergic modulators, such as benzodiazepines, given the ineffectiveness of clobazam to alter postpartum depression-like behaviors or improve maternal care. This evidence provides an important distinction that speaks to the therapeutic mechanism of action and implicates neurosteroid-sensitive, extrasynaptic $GABA_AR$s that mediate tonic inhibition, rather than benzodiazepine-sensitive synaptic receptors that mediate the phasic component of GABAergic inhibition.

Interestingly, the therapeutic effects of SGE-516 in *Gabrd*$^{-/-}$dams suggest that the effects of SGE-516 may be mediated by actions on extrasynaptic $GABA_AR$ subtypes that mediate the residual tonic current in *Gabrd*$^{-/-}$ mice. Further, the PK values suggest that the levels of SGE-516 in the brain are not high enough to activate synaptic $GABA_AR$s. These findings suggest that the therapeutic effects of SGE-516 are distinct and not shared with benzodiazepines.

In addition to the direct allosteric modulation of $GABA_AR$s, neurosteroids have also been shown to regulate $GABA_AR$s via post-translational modifications, such as phosphorylation-dependent trafficking of receptors that are in part dependent upon protein kinase C [for review see (32)]. For example, allopregnanolone and Tetrahydrodeoxycorticosterone (THDOC) have been demonstrated to increase the surface expression of $GABA_AR$s via a PKC-dependent mechanism, resulting in a sustained increase in the efficacy of GABAergic inhibition, through phosphorylation of the $GABA_AR$ α4 subunit at residue Ser443 (S443) and the $GABA_AR$ β3 subunit at Serine residues 408 and 409 (S408/9) [for review see (32)]. Therefore, SGE-516 may facilitate GABAergic inhibition independent of or in addition to the direct allosteric potentiation of $GABA_AR$ δ subunit-containing receptors, which are uniquely sensitive to neurosteroid modulation (9–12) [for review see (13, 14)]. In fact, SGE-516 has been demonstrated to induce a sustained increased in tonic GABAergic inhibition, which is associated with an increase in the phosphorylation and surface expression of the β3 subunit-containing $GABA_AR$s (33). Interestingly, although ganaxolone was demonstrated to effectively allosterically modulate $GABA_AR$s, it does not exert the sustained increase in tonic GABAergic inhibition observed with the SGE-516 compound (33). Thus, it is possible that the therapeutic effects of SGE-516 involve metabotropic regulation of $GABA_AR$s; however, further studies are required to determine whether this mechanism underlies the therapeutic effects of SGE-516 in preclinical PPD models.

In addition, our previous studies implicated HPA axis dysfunction in contributing to the abnormal postpartum phenotype in both *Gabrd*$^{-/-}$ and KCC2/Crh mice (10, 20). Data presented here demonstrate that SGE-516 is capable of suppressing the stress-induced activation of the HPA axis.

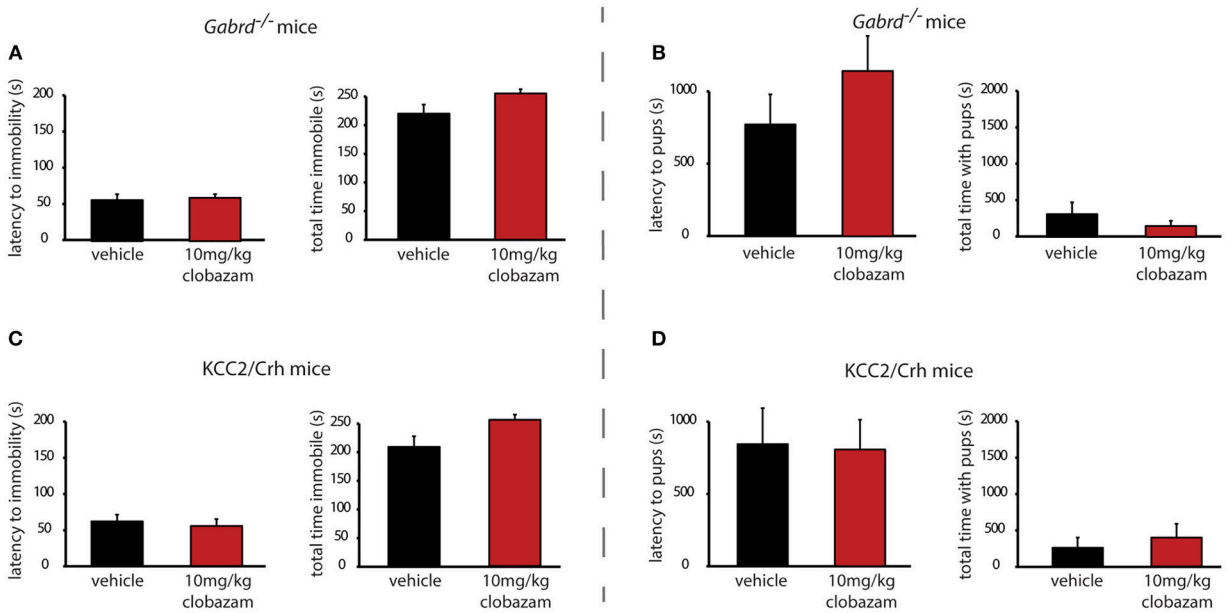

FIGURE 5 | Acute clobazam is ineffective at altering depression-like behaviors or improving maternal care in preclinical PPD models. Acute clobazam treatment (10 mg/kg chow, 30 min prior to testing) does not alter the average latency to immobility or the total time spent immobile in postpartum *Gabrd*$^{-/-}$ **(A)** or KCC2/Crh dams **(B)** in the forced swim test. Acute treatment with clobazam (10 mg/kg chow, 30 min prior to testing) does not alter the average latency to approach or the total pup interaction time in postpartum *Gabrd*$^{-/-}$ **(C)** or KCC2/Crh dams **(D)** in the maternal approach test.

However, we still do not fully understand how the HPA axis is regulated during the peripartum period and, therefore, the impact of SGE-516 in the regulation of the HPA axis during this period is also unclear. The current study demonstrates that SGE-516 treatment suppresses the stress-induced activation of the HPA axis via maintenance of KCC2 phosphorylation at residue Ser940 and total KCC2 expression in the PVN, suggesting a potential role in the peripartum regulation of the HPA axis. Previous studies implicate neurosteroids in the suppression of the HPA axis during the postpartum period (34–36) and are thought to involve a GABAergic mechanism (34, 37). We recently demonstrated that the suppression of the stress-induced activation of the HPA axis during the peripartum period involves a KCC2-dependent mechanism (20). The stress-induced dephosphorylation of KCC2 at residue Ser940 and downregulation of KCC2 in the PVN is prevented during the peripartum period (20), which we propose is critical for peripartum stress hyporeactivity that is an essential neuroendocrine adaptation, reducing vulnerability for maladaptive postpartum behaviors. It remains unclear how KCC2 is regulated in the PVN during the peripartum period, but it is possible that neurosteroids may play a role, which is an active area of ongoing research. In support of this hypothesis, here we demonstrate that SGE-516 treatment is capable of preventing the stress-induced dephosphorylation and downregulation of KCC2 and attenuates the stress-induced elevations in corticosterone levels.

Importantly, we have now established preclinical models of PPD useful for the screening of therapeutic compounds. The findings presented here are important because they demonstrate the therapeutic effectiveness of SGE-516 in several relevant preclinical models of postpartum depression. There are very few well-controlled studies that have compared the effectiveness of different classes of compounds for the treatment of PPD preclinically or clinically [for review see (38, 39)]. This study takes the first steps in comparing the ability of different classes of GABA receptor modulators to improve postpartum behaviors in preclinical models. Patients with postpartum depression are typically treated with either selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs) and there is no evidence that these treatments target the underlying biology of the disorder. The therapeutic effectiveness of SGE-516 in these preclinical PPD models is consistent with the positive clinical trial results using brexanolone for the treatment of PPD (21, 22). Further, these findings validate the use of these preclinical PPD models for screening and comparison of compounds for the treatment of PPD.

AUTHOR CONTRIBUTIONS

LM and JM were involved in conducting the experiments and data analysis included in the manuscript. LM, JM, RH, and ML were all involved in study design and interpretation of the results. JM wrote the manuscript with input from LM, RH, and ML.

FUNDING

JM is supported by NIH-NINDS grant R01 NS073574 (JM) and NS102937 (JM). LM is supported by

NIH-NIGMS grant K12GM074869; an IRACDA postdoctoral training grant to Tufts University, Training in Education and Critical Research Skills (TEACRS).

ACKNOWLEDGMENTS

The phospho-specific KCC2 antibody was a generous gift from Dr. Steve Moss (Tufts University School of Medicine).

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Conflict of Interest Statement: JM serves on the Scientific Advisory Board for SAGE Therapeutics and receives financial support for research related to the current study. RH and ML are both employees of SAGE Therapeutics.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

BRIEF RESEARCH REPORT
published: 16 July 2019
doi: 10.3389/fpsyg.2019.01033

The Role of Allopregnanolone in Pregnancy in Predicting Postpartum Anxiety Symptoms

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Postpartum depression is a serious illness affecting up to 15% of women worldwide after childbirth, and our understanding of its biology is limited. Postpartum anxiety is perhaps more prevalent and less understood. Prior studies indicate that allopregnanolone, a metabolite of progesterone, may play a role in reproductive mood disorders, including postpartum depression, but the exact nature of that role is unclear. Our own prior study in a group of psychiatrically ill women found that low allopregnanolone in the second trimester predicted the development of postpartum depression. In the present study, in both healthy and mood- and anxiety-disordered women who remained well throughout the perinatal period, we found that second trimester allopregnanolone predicted postpartum anxiety symptoms, with a similar trend toward the prediction of postpartum depressive symptoms (though without statistical significance). Both concurrent sleep and prior histories of mood and anxiety disorders contributed to the variance in mood and anxiety scores at 6 weeks postpartum. These findings confirm the importance of pregnancy allopregnanolone in postpartum psychiatric symptoms and point to future directions that may determine other important contributing factors.

Keywords: pregnancy, postpartum, allopregnanolone, hormones, depression, anxiety

OPEN ACCESS

Edited by:
Beate Ditzen, Heidelberg University Hospital, Germany

Reviewed by:
Thorsten Mikoteit, Universität Basel, Switzerland
Marlene Sophie Penz, Dresden University of Technology, Germany

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Specialty section:
This article was submitted to Psychopathology, a section of the journal Frontiers in Psychology

Received: *26 November 2018*
Accepted: *23 April 2019*
Published: *16 July 2019*

Citation:
Osborne LM, Betz JF, Yenokyan G, Standeven LR and Payne JL (2019) The Role of Allopregnanolone in Pregnancy in Predicting Postpartum Anxiety Symptoms. Front. Psychol. 10:1033. doi: 10.3389/fpsyg.2019.01033

INTRODUCTION

Postpartum depression (PPD) is a serious illness affecting up to 15% of women worldwide after childbirth (Yonkers et al., 2011), with higher rates in populations with significant psychosocial stressors. The most serious consequence of PPD, suicide, is a leading cause of maternal death in the first year postpartum worldwide (Shadigian and Bauer, 2005; Storm et al., 2014; Iacobucci, 2016; Khalifeh et al., 2016; Metz et al., 2016). Less devastating but nonetheless serious effects include poor mother-infant bonding and effects on both cognitive and emotional development in the child (Stein et al., 2014; Netsi et al., 2018). Postpartum anxiety is equally, if not more, prevalent, and is less studied and understood (Dennis et al., 2017). The timing of symptoms in vulnerable women is coincident with the abrupt withdrawal from pregnancy levels of estrogen

and progesterone at parturition (Halbreich and Kahn, 2001; Wisner et al., 2015)—but just what makes these women vulnerable is still unknown. Most studies have not found a relationship between absolute levels of hormones or the degree of decrease in levels (Heidrich et al., 1994; Harris et al., 1996; Chatzicharalampous et al., 2011; Schiller et al., 2015; Yim et al., 2015) and the development of PPD, indicating that individual women's vulnerability to the change in hormone levels is likely more important than differences in absolute levels.

Recently, there has been considerable interest in the role of allopregnanolone (ALLO), a 3α-reduced metabolite of progesterone that is a potent allosteric modulator of the GABA-A receptor and may be responsible for the neuroprotective, anxiolytic, and sedative properties of progesterone (Schule et al., 2014). Some studies (Hellgren et al., 2014; Crowley et al., 2016) have found associations between lower levels of ALLO and mood in the perinatal period, but others have not found a relationship (Deligiannidis et al., 2013). Confusingly, this is opposite to the relationship found in premenstrual dysphoric disorder (PMDD), where numerous studies have found that elevated levels of ALLO in the luteal phase are associated with increased mood symptoms (Girdler et al., 2001; Martinez et al., 2016; Timby et al., 2016), leading some to suggest that mood and anxiety responses to ALLO may follow an inverted U-shaped curve, with both low and high levels being anxiogenic and a "sweet spot" in the middle being anxiolytic (Backstrom et al., 2014).

Our group has sought to examine the relationship between mood and ALLO across the perinatal period. In a prior study, we showed that lower levels of ALLO at the second trimester of pregnancy (T2) predicted the development of a postpartum depression, with each additional ng/ml of ALLO reducing the odds of PPD by 62% (95% *CI* = 13–84%, $p = 0.022$) (Osborne et al., 2017). That study was conducted in a population of psychiatrically ill women, all of whom had a history of a mood disorder, most of whom remained on antidepressants throughout the study, and half of whom developed PPD. We were not certain whether our results would be generalizable to a less ill population and therefore sought to examine a similar question [whether T2 ALLO can predict depressive or anxious symptoms at 6 weeks postpartum (W6)] in a different population – one that is roughly equally divided among women with and without histories of mood and/or anxiety disorders, with almost all women (regardless of history) remaining psychiatrically well throughout the perinatal period.

MATERIALS AND METHODS

General Study Procedures

This was a prospective study conducted at The Johns Hopkins University School of Medicine in Baltimore, Maryland, USA. The study was approved by the Institutional Review Board of The Johns Hopkins University. We recruited both women with preexisting mood and anxiety disorders and healthy controls. Prior history of mood or anxiety disorder was determined by a thorough psychiatric interview conducted by an experienced perinatal psychiatrist, using DSM-IV criteria. Participants ($N = 124$) could enroll at any point in pregnancy, were seen for study visits at each trimester and at 6 weeks postpartum, and were managed clinically by their treating psychiatrists. Data collection included the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987) for depressive symptoms and both the Spielberg State-Trait Anxiety Inventory (STAI) (Version Y) (Ramanaiah et al., 1983) and the Perinatal Anxiety Screening Scale (PASS) (Somerville et al., 2014) for anxiety symptoms, measures of stress, baseline clinical diagnoses by the Structured Clinical Interview for DSM-IV diagnoses (administered by a trained research assistant), as well as by psychiatrist interview, personality measures, sleep quality measures [Pittsburgh Sleep Quality Index (PSQI)] (Buysse et al., 1989), medication use, and a blood draw for biological factors.

Hormone Analysis

Participant blood was collected at each visit in four 10-ml EDTA tubes. Blood samples were nonfasting, and collection times were arranged at the convenience of the participant. All occurred during the working day (9:00 a.m. to 5:00 p.m.). Samples were immediately centrifuged at 4°C for 30 min. The plasma was then aliquotted in 2 ml microcentrifuge tubes, snap frozen on dry ice, and immediately stored in a −80°C freezer. Blood was analyzed with the allopregnanolone EIA kit from Arbor Assays LLC Cat 3 KC44-H1. All samples were run in duplicate, and the coefficient of variation (CV) among samples was <10%. Predicted hormone concentrations were reshaped and merged with participant IDs prior to analysis.

Statistical Analysis

Participants were included in the analytic cohort if they had both a blood sample at T2 and completed psychological scales at W6. Twenty-four subjects (19.4%) enrolled in the study after T2 and so did not have a blood draw at T2. Seven participants (5.6%) did not complete any psychological scales at W6, and one participant (0.8%) was missing covariates, leaving a total of 92 participants in the analytic cohort. Negative Binomial Generalized Linear Models (GLMs) were used to explore relationships between the log of ALLO in the second trimester and week 6 postpartum psychiatric outcomes (as measured by the EPDS, STATE score of the STAI, and PASS). Models were adjusted for maternal age, sleep (PSQI Global Score), and timing of week 6 postpartum visit. The relationship with ALLO was additionally explored by history of mood disorder, history of anxiety disorder, and history of psychiatric medication usage in three separate models. Each model included a main effect of the variable, as well as an interaction between the variable and log ALLO. Model assumptions were assessed using residual diagnostic plots. All continuous covariates (maternal age, visit timing, PSQI) were centered at their respective sample means. Robust standard errors were used in hypothesis tests and to construct 95% confidence intervals. Statistical analyses were performed using R version 3.5.1[1].

[1]https://www.R-project.org

RESULTS

Ninety-two women met criteria for inclusion in these analyses. Of those women excluded from analysis, 65.6% were white (compared to 89.1% of those included, p = 0.0049) and 78.1% were married (compared to 94% of those included, p = 0.013); there were no other significant differences between groups. The average age of the women included was 32.5 (*SD* = 3.7), and they were highly educated (with 61.5% having attained a graduate or professional degree); 21.4% of those with available medication history used psychiatric medications during the index pregnancy. The majority (58.7%) had a mood disorder, and 35.9% had an anxiety disorder. Mood disorder diagnoses broke down as 3.3% with bipolar I disorder; 3.3% with bipolar II disorder; and 47.8% with major depressive disorder. Complete demographic features of the entire sample, as well as those included and excluded in the analysis, are reported in **Table 1**. Most women, including those with mood disorders, remained psychiatrically well throughout the study. The median EPDS score at T2 was 4 (IQR 2, 7), at T3 4 (IQR 1, 7), and at W6 3 (IQR 1, 7), with few women attaining a score above the clinically meaningful cutoff of ≥13, indicating possible depression (5 at T2, 5 at T3, and 4 at W6). Median STAI State scores were 29 (IQR 23.8, 35) at T2, 29 (IQR 23, 37) at T3, and 27 (IQR 22, 39) at W6. Median PASS scores were 10 (IQR 5, 18.2), 10 (IQR 4.5, 18), and 10 (IQR 4.8, 18). There were more women with anxiety scores above a clinically meaningful cutoff (>35 on the STAI and ≥21 on the PASS), with 25 at T2, 27 at T3, and 30 at W6 for STAI and 16 at T2, 13 at T3, and 19 at W6 for the PASS. Mean log of ALLO levels were 1.6 at T2 (*SD* = 0.5), 2.2 at T3 (*SD* = 0.6), and −0.5 at W6 (*SD* = 0.6).

TABLE 1 | Demographic characteristics of participants.

Variable	All (*N* = 124)	Included (*N* = 92)	Excluded (*N* = 32)
Mean age (*SD*)	32.6 (3.7)	32.5 (3.7)	32.9 (3.8)
Race			
White	103 (83.1%)	82 (89.1%)	21 (65.6%)
Black	11 (8.9%)	5 (5.4%)	6 (18.8%)
Asian/Pacific Islander	7 (5.6%)	3 (3.3%)	4 (12.5%)
Relationship status			
Single	5 (4%)	2 (2.2%)	3 (9.4%)
Married	112 (90.3%)	87 (94%)	25 (78.1%)
Widowed	0 (0%)	0 (0%)	0 (0%)
Cohabiting	6 (4.8%)	3 (3.3%)	3 (9.4%)
Education			
High school graduate	4 (3.3%)	2 (2.2%)	2 (6.2%)
Some college	7 (5.7%)	5 (5.5%)	3 (6.2%)
Bachelor's degree	28 (22.8%)	22 (24.2%)	6 (18.8%)
Some graduate	8 (6.5%)	6 (6.6%)	2 (6.3%)
Graduate degree	76 (61.8%)	56 (61.5%)	20 (62.5%)
History mood disorder	76 (61.3%)	54 (58.7%)	22 (68.8%)
History anxiety disorder	47 (37.9%)	33 (35.9%)	14 (43.8%)
Taking psychiatric meds	24 (21.8%)	18 (21.4%)	6 (23.1%)
T2 ALLO (*SD*), ng/ml	5.2 (2.7)	5.3 (2.7)	4 (1.1)
Log T2 ALLO (*SD*)	1.5 (0.5)	1.6 (0.5)	1.4 (0.3)
W6 EPDS (IQR)	4 (1, 8)	3 (1, 5.8)	4 (2, 9)
W6 STATE (IQR)	27 (21, 31.5)	27 (22, 39)	25.5 (21, 33.8)
W6 PASS (IQR)	4 (2, 6)	4 (2, 6)	5 (2.5, 6)

T2 = second trimester; ALLO = allopregnanolone; W6 = 6 weeks postpartum; EPDS = Edinburgh Postnatal Depression Scale; STATE = State score of the Spielberg State – Trait Anxiety Inventory, Version Y Totals do not equal 100% due to missing data.

As seen in **Figure 1,** both EPDS and PASS scores in week 6 exhibited a negative association with log ALLO in the second trimester, with lower log ALLO at T2 being associated with higher symptom scores at W6; STAI scores exhibited a flatter trend. After adjusting for maternal age, gestational age, and sleep quality, higher log ALLO in the 2nd trimester was associated with lower EPDS and PASS scores (~30% lower scores per 1 unit increase in log ALLO in both outcomes), but STAI-State scores did not exhibit an association. Only the aggregate association between PASS and log ALLO was statistically significant at the 5% level (exponentiated β = 0.68, 95% *CI* = 0.48–0.97, p = 0.025). Concurrent sleep quality also accounted for some of the variation in W6 scores, with higher PSQI scores, indicating poorer sleep quality, related to higher symptoms on all inventories. All other things being equal, each unit increase in global PSQI score at W6 was associated with a 10% increase in the concurrent EPDS score (exponentiated β = 1.10, 95% *CI* = 1.02–1.17, p = 0.005), a nonsignificant 3% increase in the concurrent STATE score (exponentiated β = 1.03, 95% *CI* = 0.99–1.06, p = 0.073), and an 14% increase in the concurrent PASS score (exponentiated β = 1.14, 95% *CI* = 1.08–1.21, $p < 0.001$).

We then allowed relationships with ALLO in the regression models to vary by history of mood disorder, history of anxiety disorder, and concurrent medication use. In the adjusted model with an interaction, a history of mood disorder was associated with a 2.4-fold increase in the EPDS score at W6 compared to no history of mood disorder at the average level of log ALLO (estimated means at the reference level of all variables: no history of mood disorder = 1.99, 95% *CI* = 1.12–3.54; with history of mood disorder = 4.80, 95% *CI* = 3.81–6.06; exponentiated β = 2.41, 95% *CI* = 1.35–4.30, p = 0.003) and a 1.8-fold increase in the PASS score at W6 (estimated means at the reference level of all variables: no history of mood disorder = 8.06, 95% *CI* = 4.52–14.35; with history of mood disorder = 14.53, 95% *CI* = 11.51–18.33; exponentiated β = 1.80, 95% *CI* = 1.01–3.21, p = 0.045). The interaction term that included history of mood disorder and T2 log ALLO was statistically significant in the model for EPDS score (exponentiated β = 0.36, 95% *CI* = 0.14–0.90, p = 0.018), indicating opposite trends for the relationship of T2 ALLO to W6 scores depending on history of mood disorder diagnosis, although these trends did not reach statistical significance at the 5% level. Those who had no history of a mood disorder had a 90% increase in EPDS scores (exponentiated β = 1.94, 95% *CI* = 0.96–3.90, p = 0.065) for each log unit increase in T2 ALLO, while

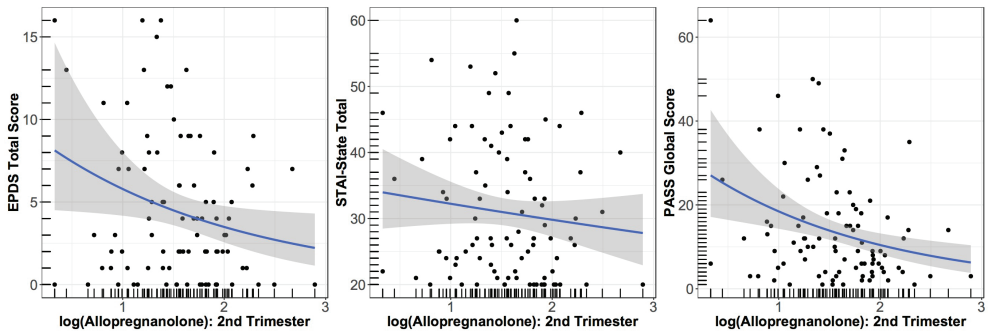

FIGURE 1 | Relationship between EPDS score at W6 and log of ALLO concentration at T2 (p = 0.10); between STATE score at W6 and log of ALLO concentration at T2 (p = 0.55); and between PASS score at W6 and log of ALLO concentration at T2 (p = 0.025). Smoothed averages are shown from a negative binomial generalized additive model, with 95% confidence interval.

those who did have a history of a mood disorder had a 31% decrease in EPDS score (exponentiated β = 0.69, 95% *CI* = 0.43 to 1.10, p = 0.122) for each log unit increase in T2 ALLO. History of anxiety disorder did not affect the directionality of these relationships. In the adjusted model with interaction, history of anxiety disorder at the average level of T2 log-ALLO was associated with a 1.9-fold increase in EPDS at W6 (exponentiated β = 1.90, 95% *CI*, 1.33–2.73, p = 0.001) and a 1.7-fold increase in the PASS score at W6 (exponentiated β = 1.73, 95% *CI* = 1.21–2.49, p = 0.003). Concurrent medication use was not a significant effect modifier and did not have a statistically significant effect on W6 scores at the average level of T2 log-ALLO.

DISCUSSION

In a population of women with and without mood and anxiety disorders, almost all of whom remained psychiatrically well throughout the study, we found an association between lower T2 ALLO and higher anxiety scores at 6 weeks postpartum, as well as a similar relationship between T2 ALLO and higher W6 depressive symptoms, which did not reach statistical significance in the adjusted model. While this does not exactly replicate our previous findings, the intriguing additional finding of an opposite relationship between T2 ALLO and W6 mood scores depending on prior history of mood disorder may indicate that women with mood disorders may respond differently to allopregnanolone than those without. We also found that, controlling for the level of ALLO at T2, each additional increment of disrupted sleep at W6 (as measured by a one-point increase in the global PSQI score) was associated with an additional 10% increase in depressive symptoms and 11% increase in anxiety symptoms, and that women with histories of prior mood or anxiety disorders had a roughly two-fold increase in both EPDS and PASS scores in the postpartum compared to those with no history at the average level of T2 ALLO (in keeping with the amount that prior literature has found to represent a clinically important increase) (Matthey, 2004).

Interestingly, our findings depended upon the tool used to measure anxiety. The relationship between pregnancy allopregnanolone and postpartum anxiety was detectable only with an instrument designed specifically for the perinatal population, the PASS; we did not detect a relationship when anxiety was measured with an instrument designed for the general population (the STAI).

Several factors may underlie the differences between this and our prior study. The prior study included measures of clinician-diagnosed depression, whereas this study used EPDS scores as a proxy for postpartum depressive symptoms; the two may not be comparable, and the EPDS, while well validated as a screening tool, is not a diagnostic tool (Cox, 2017). In the current population, at T2, only 5.4% had EPDS scores ≥13, indicative of possible depression, and only 4.3% were above that cutoff at 6 weeks postpartum. By contrast, in our prior study, 38% were depressed (by clinician diagnosis using DSM-IV criteria) at T2 and 48% at W6. As an additional indication of severity of illness, 21.4% of the subjects in the current study were using psychiatric medications, while 74% of the population in the prior study was on medications.

While T2 ALLO was not statistically significantly associated with W6 EPDS score, the point estimate and shape of the relationship were identical to those found in our prior study. In addition, the difference we found between women with and without a history of mood disorder (with those with history showing higher W6 EPDS scores for lower T2 ALLO, and those without history showing the opposite) may indicate that this relationship holds only for women with mood disorders. In our prior study, we were not able to assess anxiety symptoms; the association we found here between T2 ALLO and W6 anxiety symptoms may indicate that ALLO's predictive value for PPD exists because of its effects on anxiety symptoms (as anxiety is a major clinical feature of PPD). In addition, we have shown a substantial effect of sleep, perhaps indicating that the path to anxiety and depression from ALLO may lie through poor sleep (or, conversely, that those with poor sleep may have lower levels of ALLO).

This is an exploratory study, and as such, there are substantial limitations. The sample size is small (though larger than that in our previous study), and most participants were highly educated white women. We were unable to control for some clinical confounders that could have affected our results, including body mass index, levels of other hormones, and other medical conditions. Blood was not collected at the same time point during the day for each subject, and it is possible that our results were affected by diurnal variations. We did not collect information about fetal sex and so were unable to examine any differences in mood, anxiety, or hormone level by sex of the fetus.

These results nevertheless indicate that allopregnanolone early in pregnancy continues to be an intriguing player in postpartum mood and anxiety symptoms; further studies on exactly how that relationship may work (what are the additional steps in the chain between second trimester ALLO and postpartum symptoms?) will prove a rich area of research. In addition, our work shows that sleep and especially prior history are also substantial independent factors. This should be good news for our field, as sleep interventions and careful screening for prior depressive episodes and/or anxiety disorders are low-cost tools that should be easy to implement and could make substantial improvements in our ability to prevent postpartum depression.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Institutional Review Board of the Johns Hopkins University with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of the Johns Hopkins University.

AUTHOR CONTRIBUTIONS

LMO collected data and wrote the paper. JLP and LRS collected data and edited the paper. JFB and GY designed and carried out statistical analyses.

FUNDING

LMO was supported in this work by the National Institute of Mental Health, 1K23 MH110607, the Brain and Behavior Foundation Young Investigator Award, and the Doris Duke Early Clinician Investigator Award. This work was also supported by National Institute of Mental Health 1R01MH112704-01.

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Conflict of Interest Statement: JLP receives research support from Sage Therapeutics and holds a patent for epigenetic biomarkers of postpartum depression.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 12 June 2019
doi: 10.3389/fpsyg.2019.01273

The Impact of Parental Role Distributions, Work Participation, and Stress Factors on Family Health-Related Outcomes: Study Protocol of the Prospective Multi-Method Cohort "Dresden Study on Parenting, Work, and Mental Health" (DREAM)

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OPEN ACCESS

Edited by:
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Reviewed by:
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OsloMet – Oslo Metropolitan University, Norway
Berta Rodrigues Maia,
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Specialty section:
This article was submitted to Health Psychology, a section of the journal Frontiers in Psychology

Received: 15 November 2018
Accepted: 14 May 2019
Published: 12 June 2019

Citation:
Kress V, Steudte-Schmiedgen S, Kopp M, Förster A, Altus C, Schier C, Wimberger P, Kirschbaum C, von Soest T, Weidner K and Junge-Hoffmeister J and Garthus-Niegel S (2019) The Impact of Parental Role Distributions, Work Participation, and Stress Factors on Family Health-Related Outcomes: Study Protocol of the Prospective Multi-Method Cohort "Dresden Study on Parenting, Work, and Mental Health" (DREAM). Front. Psychol. 10:1273. doi: 10.3389/fpsyg.2019.01273

The Dresden Study on Parenting, Work, and Mental Health ("**DR**esdner Studie zu **E**lternschaft, **A**rbeit, und **M**entaler Gesundheit", **DREAM**) aims to prospectively investigate the relationship between parental work participation, role distribution, stress factors, and their effects on perinatal outcomes and long-term family mental and somatic health in a community sample targeting $N = 4{,}000$ individuals, i.e., 2,000 couples, expecting a child and residing in Dresden, Germany (interim sample of $N = 1{,}410$ participants, recruitment ongoing). Various questionnaires are completed at four measurement points from pregnancy to 2 years postpartum (prolongation into middle childhood planned). Applying a multi-method approach, long-term endocrinological data (analyses of hair cortisol concentrations and other endogenous hormones, "$\text{DREAM}_{\text{HAIR}}$") and qualitative interview data (regarding gender role attitudes and distribution of domestic work, child care, and paid employment; "$\text{DREAM}_{\text{TALK}}$") are obtained. In this study protocol, the theoretical background, methods, and preliminary results considering sociodemographic characteristics during pregnancy and birth-related factors at 8 weeks postpartum are presented. Additionally, there is a focus on our endocrinological sub-study $\text{DREAM}_{\text{HAIR}}$. In this sub-study currently comprising $N = 152$ participants, i.e., 88 families (recruitment ongoing), we want to gain knowledge on the transgenerational processes of stress regulation and psychopathology in the whole family by analyzing hair cortisol concentrations in both parents and children during the course

from pregnancy (or after birth regarding children) to at least 2 years postpartum. By comparing data of the community sample to a clinical sample of mothers with postpartum mental disorders, their children, and their partners during the period between admission and discharge from a mother-baby unit and post-treatment ("$DREAM_{MBU}$"), the course of mothers' psychopathology, parent-infant interaction, and infant regulation disorders with special regard to long-term endocrine correlates will be examined. With previous studies neglecting the fathers or partners involved, a major advantage of DREAM is the use of a multi-method and multi-level approach by examining the whole family in a longitudinal design. Therefore, the DREAM study will contribute to a better understanding of the role of social, work, and stress factors for mental and somatic health and its long-term endocrine correlates in the natural course of becoming a family.

Keywords: parental mental health, work participation, role distribution, peripartum stress, DREAM study, hair cortisol, multi-method approach, study protocol

INTRODUCTION

Expecting a child marks a transition involving several physiological, psychological, and structural changes for the individual as well as for the couple. The majority of German women reduces its work participation after giving birth to a child and continues staying at home after paid parental leave runs out 12 months postpartum (or after the stretched out parental leave ends 2 years postpartum for families who worked part-time for a while); and of those women who do return to their jobs, the majority only works part-time (Bundesministerium für Familie Senioren Frauen und Jugend, 2017a). German mothers are eligible to split the parental leave with the father and if the fathers take at least two so-called "fathers' months" of paid leave, the parental allowance is extended to 14 months in total. Although the amount of fathers taking paternal leave is increasing (with roughly one third taking the "fathers' months"), men usually stay at home for a shorter period compared to women and continue working the same amount of time afterwards (Bundesministerium für Familie Senioren Frauen und Jugend, 2017a). This imbalance is aggravated by several cultural reasons (e.g., when it is appropriate to work again and concerning distribution of labor between sexes) and structural reasons like the tax system (i.e., the German tax system penalizes married couples economically when both partners share domestic work, child care, and paid employment equally, leading to a polarization of working time), a cash-for-care benefit ("Betreuungsgeld"; which subsidies parents (mostly women) staying at home with their children until they turn 3 years old), and an insufficient access to day care. Short- and long-term consequences of mothers' longer parental leave and not being employed refer to an unbalanced distribution of domestic work and child care (Buehler and O'Brien, 2011; Schober and Zoch, 2015), unequal wages after re-entry in the labor market (Davies and Pierre, 2005; Bryan and Sevilla-Sanz, 2010), and a delay in their professional career which may result in an increasing dependency on the partner and consolidate a gender gap interfering with gender equality (Barker and Pawlak, 2011; Miani and Hoorens, 2014).

Beyond equality considerations, the impact of paid employment on health has to be considered. In this context two contrary hypotheses have been suggested: the role strain or scarcity hypothesis postulates that additional roles like being a working woman impair maternal health due to additional daily hassles and demands (Goode, 1960). Thus, it may be more challenging to care for oneself and for a child and meet its needs. In contrast, the role enhancement hypothesis suggests that women with several roles are healthier because of positive stimulating input in their professional life and better access to resources that help in dealing with these demands (Sieber, 1974; Marks, 1977). As a consequence, the mother may have more energy to dedicate herself to family life in her spare time. In accordance with the latter, there is recent evidence that maternal work participation may have a positive influence on mental and somatic health (Klumb and Lampert, 2004; Buehler and O'Brien, 2011; Frech and Damaske, 2012; Cruise et al., 2018). Nevertheless, further results suggest a negative (in line with the scarcity hypothesis) or missing association between maternal work participation and the mother's mental and somatic health (Schwab-Reese et al., 2017; Liu et al., 2018). Consistent with findings on negative associations, women have been found to have a greater risk for suffering from work-privacy conflict than men probably due to multiple private and occupational burdens (Garthus-Niegel et al., 2016). Thus, it may be hard to put compatibility of work and family into practice. In sum, the potential etiologic role of employment on health warrants further investigation. Indeed, as the above mentioned evidence mainly comes from cross-sectional studies conducted in the United States, it remains unclear whether the previous research results are applicable to working women and families in Europe or even Germany, where maternal work participation is comparatively low. Moreover, previous evidence also neglected the role of important confounding or moderating factors, e.g., precarious working conditions and psychosocial work stress (Klumb and Lampert, 2004; Buehler and O'Brien, 2011), despite of recent evidence indicating their negative impact on parents' mental and somatic health (Caparros-Gonzalez et al., 2017; Philpott et al., 2017).

Chronic effects of stress on mental and somatic health, e.g., caused by precarious working conditions or psychosocial work stress, have been closely linked to the activity of the body's stress response systems, particularly the hypothalamic-pituitary-adrenal (HPA) axis leading to the secretion of cortisol (Chrousos, 2009). Studies investigating the relationship between work participation or precarious working conditions and cortisol are predominantly based on traditional cortisol measures. For example, unemployment has been found to be linked with alteration of diurnal or overall cortisol secretion, e.g., in blood (e.g., Arnetz et al., 1991; Maier et al., 2006) or saliva (e.g., Grossi et al., 2001; Gallagher et al., 2016). The direction of findings is characterized by some inconsistency (review: Sumner and Gallagher, 2017), e.g., with some studies indicating elevated (e.g., Arnetz et al., 1991) or lowered (e.g., Gallagher et al., 2016) overall cortisol levels in unemployed people compared to employed people, while some studies failed to show such a difference (e.g., Ockenfels et al., 1995). Regarding the relations between psychosocial work stress on the activity of the HPA axis, study results support the relevance of endocrine correlates as a mediator of the impact of work stress on health although findings are characterized by a notable heterogeneity (review: Siegrist and Li, 2017). Part of the reason for mixed results may be due to limitations in the assessment of long-term cortisol secretion. Specifically, previous cortisol assessment methods particularly reflect short-term secretory activity over periods ranging from minutes (saliva, plasma) to hours (urine; Stalder et al., 2017). Given that acute cortisol secretion is highly volatile and affected by a range of situational factors (Stalder and Kirschbaum, 2012), these methods provide rather unreliable estimates of long-term cortisol output. The analysis of hair cortisol concentrations (HCC) constitutes a relatively recent tool that may increase the quality of the assessments of long-term cumulative cortisol levels in such research. Through an incorporation of lipophilic substances into the slowly growing hair matrix, HCC are supposed to be a non-invasive and easily obtainable retrospective marker of cortisol levels integrated over the previous months (reviews: Stalder and Kirschbaum, 2012; Stalder et al., 2017). Further advantages of hair cortisol analysis include the robustness to acute situational influences and the independency from non-compliance issues (Russell et al., 2012; Stalder and Kirschbaum, 2012; Stalder et al., 2017). Over the past years, considerable evidence has emerged in support of the general validity and reliability of hair cortisol analysis (review: Stalder and Kirschbaum, 2012). Specifically, recent studies have shown positive associations between HCC and cumulative cortisol data from repeated assessments using traditional cortisol measures (Sauvé et al., 2007; D'Anna-Hernandez et al., 2011; van Holland et al., 2012; Short et al., 2016). Further indirect support stems from research that found a correspondence of HCC data and the expected secretory pattern in conditions with well-known endocrine alterations (review: Stalder et al., 2017). For example, the well-known pattern of increasing cortisol levels over the course of pregnancy was confirmed by HCC data (Kirschbaum et al., 2009; D'Anna-Hernandez et al., 2011; Karlén et al., 2013; Hoffman et al., 2016).

However, evidence for HCC in relation to work participation and precarious working conditions remains rare and inconsistent. Some studies indicate links between HCC and unemployment (Dettenborn et al., 2010), job insecurity (Herr et al., 2017), and shift work (Manenschijn et al., 2011). Other studies have come to opposite findings underlining the need of further investigations on larger samples taking into account moderating and mediating factors within a longitudinal design (Janssens et al., 2017; van der Meij et al., 2018). Furthermore, HCC were found to serve as a marker of stress in several contexts, e.g., stress-related somatic conditions (Pereg et al., 2011; Manenschijn et al., 2013; Stalder et al., 2013; Kuehl et al., 2015) and mental disorders (review: Staufenbiel et al., 2013; Wester and van Rossum, 2015; Stalder et al., 2017; Steudte-Schmiedgen et al., 2017). Evidence suggests that HCC sensitively reflect clinical and/or stress-related conditions. For example, higher HCC were detected in patients with major depression (Dettenborn et al., 2012; Hinkelmann et al., 2013; Wei et al., 2015) and late-onset bipolar disorder (Manenschijn et al., 2012) while attenuation was seen in generalized anxiety disorder (Steudte et al., 2011) or posttraumatic stress disorder characterized by a long-term time-interval since traumatization (Steudte et al., 2013). Still, meta-analytic data revealed no consistent relationships with questionnaire-based measures of perceived stress or clinical symptoms (Stalder et al., 2017). This seems also to be evident among pregnant women, i.e., HCC were not consistently found to be related to prenatal psychological distress (review: Mustonen et al., 2018). Interestingly, Bowers et al. (2018) found an association between self-reports of distress and HCC during pregnancy only in women who experienced high levels of childhood adversity compared to women without such experiences. This supports the notion that early adversity contributes to the long-term activity of the HPA axis. Here, a longitudinal investigation of HCC over an extended period of time is warranted. However, the fact that HCC were more frequently related to the number of self-reported stressful or negative life events (Karlén et al., 2011, 2015; Grassi-Oliveira et al., 2012; Staufenbiel et al., 2014; Steudte-Schmiedgen et al., 2015) or traumatic events across the lifespan (Steudte et al., 2013; Steudte-Schmiedgen et al., 2016) suggests that stronger psychoendocrine correspondence may be achieved by using stress measures based on objectives criteria.

So far, only implications for the mother's health herself have been considered in this paper. More importantly, stress factors, e.g., precarious working conditions and psychosocial work stress, may not only be essential for the health of the mother herself, but also play a major role for the entire family and especially for development and health of the offspring (Lucas-Thompson et al., 2010; Jaursch and Lösel, 2011). In fact, the early environment is fundamental for long-term mental and somatic health of a child (Van den Bergh et al., 2017). Child outcomes have been found to be negatively affected by maternal mental disorders (Pearson et al., 2013; Gentile, 2017) and self-reported stress (Wadhwa et al., 2011; Van den Bergh et al., 2017) during pregnancy.

Long-term effects of maternal adversity on the offspring are referred to as fetal programming with a transgenerational transmission of alterations of the long-term activity of the HPA

(Räikkönen et al., 2011; Beijers et al., 2014). Thus, cortisol has been considered as an important mediator of the effects of maternal stress on the developing brain of the fetus (review: Entringer et al., 2015). Specifically, this effect is assumed to be regulated by the placental enzyme 11 beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) which converts cortisol in its inactive form cortisone (Beitins et al., 1973; Brown et al., 1996). Importantly, some proportion of active maternal cortisol passes through the placenta into the fetal compartment. In line with this assumption, adverse intrauterine conditions such as maternal anxiety (O'Donnell et al., 2012), severe infection (Johnstone et al., 2005), or alcohol exposure (Liang et al., 2011) have been found to co-occur with a down-regulation of placental 11beta-HSD2 activity. Another mechanism that may regulate the fetal programming effects of elevations in maternal cortisol is increased production of placental corticotrophin-releasing hormone (CRH), which, in turn, may affect the fetal HPA axis and the biosynthesis of adrenal steroids (review: Entringer et al., 2015).

Previous evidence found several parental risk factors that may affect long-term regulation of the body's stress response systems in the offspring, e.g., low socioeconomic status (Essex et al., 2002; Evans and Kim, 2007) or maternal psychopathology (Gentile, 2017). Specifically, changes in maternal cortisol regulation due to (traumatic) stress, anxiety, or depressiveness have been shown to result in altered fetal cortisol levels as measured in saliva (Yehuda et al., 2005; Brennan et al., 2008; Grant et al., 2009; Davis et al., 2011) and urine (Yehuda et al., 2002; Diego et al., 2004). However, the reported relationships between various measures of prenatal maternal stress and offspring cortisol are complex and heterogeneous (Van den Bergh et al., 2017) which may be partly the result of the use of short-term cortisol measures as mentioned earlier. Recently, research using HCC analysis among newborns is emerging (e.g., Yamada et al., 2007; Hoffman et al., 2017) highlighting the unique potential of neonatal HCC to reflect intrauterine glucocorticoid regulation. Specifically, it has been shown that cortisol levels of neonatal hair which was taken at birth mainly reflect the third trimester increase of maternal cortisol (Hollanders et al., 2017).

Studies examining HCC in infants found associations to maternal HCC during pregnancy, supporting the notion of a transgenerational transmission of alterations of the long-term activity of the HPA axis (Karlén et al., 2013; Romero-Gonzalez et al., 2018). As mentioned above, recent studies found close links between trauma exposure across the lifespan and long-term regulation of HPA axis activity (Steudte-Schmiedgen et al., 2016; Pervanidou et al., 2017). Interestingly, initial cross-sectional HCC research supports the notion of a relationship between lifetime trauma exposure, childhood abuse, and HCC during pregnancy (Schreier et al., 2015, 2016; Swales et al., 2018). A prospective study further confirmed associations between maternal lifetime trauma history, HCC reflecting the third trimester of pregnancy, and subsequent infant negative affectivity at the age of 6 month (Bosquet Enlow et al., 2017). This is commensurate with study data observing an association between maternal lifetime trauma exposure and increased HCC in older children (i.e., at the age of three and four), albeit this relationship was not found in infants under the age of two (Slopen et al., 2018). These promising findings are in line with the assumption of an intergenerational transmission of maternal childhood maltreatment and necessitate further prospective investigations (Buss et al., 2017).

Not only intrauterine but also postpartum factors can affect long-term activity of the HPA axis of children as measured by HCC analysis. Research about the role of parent-infant interaction is slowly emerging. For example, a study found that mothers with increased HCC were more intrusive and showed lower positive engagement synchrony with their offspring 6 months after delivery (Tarullo et al., 2017). This corresponds with a study showing an association between mother's parenting stress and depressiveness at 4 weeks postpartum and higher HCC in their infants at the age of one, which, in turn, were related to pronounced socioemotional problems (Palmer et al., 2013). Thus, both intrauterine and postpartum influences on the infant HPA axis can manifest in infant health and behavior, e.g., a difficult temperament or regulatory problems that can precede later psychopathology and impair further development of the child (Gunnar and Donzella, 2002; Hemmi et al., 2011; Davis and Sandman, 2012; Stein et al., 2014; Petzoldt et al., 2016). Still, evidence regarding the relations between maternal self-reported distress, activity of the HPA axis, and infant outcomes is rare, inconsistent, and mainly stems from studies assessing short-term cortisol (e.g., in saliva: Bosquet Enlow et al., 2017; Van den Bergh et al., 2017). Hence, there is need of further investigations to detect how maternal HCC are reflected in long-term changes of health and behavior problems and possibly underlying biological stress reactions of the child indicated by neonatal HCC.

Moreover, it is of significance whether psychological intervention can support a normalization of HPA axis dysregulation in both mother and child. Over the past years, evidence emerged supporting the notion that effective psychotherapy may improve this kind of dysregulations in patients suffering from posttraumatic stress disorder. Specifically, cortisol levels were found to increase in responders to psychotherapy while those of non-responders decreased (Olff et al., 2007; Yehuda et al., 2009, 2014), albeit with some inconsistency in findings (review and meta-analysis: Gerardi et al., 2010; Pacella et al., 2014; Schumacher et al., 2018). Further, evidence has accumulated in support of the potential of pre-treatment hormone concentrations as predictors of successful psychotherapeutic outcome in patients with posttraumatic stress disorder (review: Colvonen et al., 2017) and patients with affective and/or anxiety disorders (Fischer et al., 2018). Regarding postpartum mental disorders, treatments combining psychological, psychopharmacological, and interactional components in a mother-baby unit (MBU) are found to contribute to lower psychopathology (review: Connellan et al., 2017). So far, no study has investigated the role of long-term endocrine correlates as predictor and correlate of successful clinical outcome of such a treatment among mothers and their infants. If there are alterations of the long-term activity of the HPA axis going along with the therapy, considering the linkage between maternal and infant HPA axis, it can be reasonably assumed that not only maternal health but also infant health

and behavior and its endocrine correlates may improve within treatment of postpartum mental disorders.

As mentioned above, the involvement of fathers with their children is slowly increasing (Barker and Pawlak, 2011; Bundesministerium für Familie Senioren Frauen und Jugend, 2017a). Still, the role of fathers and partners related to the mother has been widely neglected in previous studies. In particular, there is a lack of evidence with regard to the mechanisms through which the partner may have an impact on the child (Barker et al., 2017). Emerging research suggests that, in accordance to the evidence regarding mothers, early father-infant-interaction and parenting affect child development and behavior (Ramchandani et al., 2013; Parfitt et al., 2014). Consistent with this, psychopathology of the father has detrimental effects on parenting (review: Wilson and Durbin, 2010) and (maybe in turn) on child outcomes (Ramchandani et al., 2005; Sweeney and MacBeth, 2016). Regarding endocrinological aspects, recent studies indicate that couples' mental health and physiological states (especially cortisol) are associated with one another, which is assumed to have implications for health and functioning as a couple, e.g., role distribution or partnership satisfaction (Timmons et al., 2015), which, in turn, may have a direct or indirect impact on child outcomes (Hanington et al., 2012; Parfitt et al., 2014).

In conclusion, the impact of long-term effects of stress, e.g., caused by precarious working conditions or psychosocial work stress, on mental and somatic health of the whole family warrants more research. In particular, previous studies were limited to self-reported measures or short-term cortisol assessment methods (e.g., saliva or urine). Consequently, more detailed information about the HPA axis linkage between parents and in parent-child dyads is needed to examine the pathways of transgenerational transmission of long-term regulation of the HPA axis and possible approaches for intervention.

To close this gap, the current cohort study called Dresden Study on Parenting, Work, and Mental Health (**DREAM**; "**DR**esdner Studie zu **E**lternschaft, **A**rbeit und **M**entaler Gesundheit") together with its sub-studies $DREAM_{HAIR}$ and $DREAM_{TALK}$ combining quantitative questionnaires, qualitative interviews, and long-term endocrine correlates aims to examine the impact of parental work participation, role distributions, and stress factors on family health longitudinally, i.e., during the course from late pregnancy to 2 years postpartum.

Based on the theoretical model shown in **Figure 1**, the following main questions will be investigated:

I. How do mothers' and their partners' work participation and role distribution regarding domestic work, child care, and paid employment change in the course from pregnancy to 2 years postpartum; in particular, will there be a shift toward more traditional gender roles (DREAM and $DREAM_{TALK}$)?
II. How are mothers' and their partners' mental and somatic health as well as child health and behavior influenced by their work participation over time and is a potential association influenced by confounding or moderating factors such as precarious working conditions and psychosocial work stress (DREAM and $DREAM_{TALK}$)?
III. How does mothers' and their partners' mental health affect child health and behavior cross-sectionally and longitudinally (DREAM)?
IV. How are HCC inter-related between the family members (mother, father, index child) cross-sectionally and longitudinally ($DREAM_{HAIR}$)?
V. Are HCC predicted by stress factors (including precarious working conditions and psychosocial work stress)? How do HCC relate to mental health and behavioral outcomes of each family member cross-sectionally and longitudinally ($DREAM_{HAIR}$)?
VI. Regarding a clinical sample, are maternal mental disorders and long-term HCC of mothers (and partners if available) at admission to the MBU associated with behavioral outcomes and HCC of the child and how does this change during the course of psychotherapeutic treatment ($DREAM_{MBU}$)?

With its multi-method approach addressing both parents and child, this study is unique in the research on the interplay of work participation, role distribution, and stress factors and its impact on perinatal outcome and mental and somatic health of the whole family. Considering the complex relationships between prenatal and postnatal psychological distress and long-term cortisol secretion in parents and their offspring, this study will make a significant contribution to the existing literature.

METHODS AND ANALYSES

Overall Design and Procedure

The DREAM study is a prospective cohort study targeting a total community sample of $N = 4{,}000$ individuals, i.e., 2,000 couples, expecting a child in and around Dresden, Germany. Inclusion criteria are a current pregnancy, being a resident in the mentioned area, and sufficient German skills to complete the study questionnaires. In order to get a comprehensive picture of (becoming) parents in the area, there was no exclusion of comparatively rare groups, e.g., multiple pregnancies, couples of the same sex, and single persons. Since June 2017, pregnant women [subsequently referred to as (expectant) mothers], and the male or female partners they are currently involved with in a long-term relationship are recruited during pregnancy mainly in obstetrical clinics and midwife practices, with an interim sample of $N = 1{,}410$ participants who have completed the first questionnaire (T1) by the end of September 2018 (recruitment ongoing). The DREAM study has been approved by the Ethics Committee of the Faculty of Medicine of the Technische Universität Dresden (No: EK 278062015).

Following milestones for young German families, in the basic DREAM study there are four measurement points (**Figure 2**) starting with T1 during pregnancy and three postpartum assessment waves, i.e., T2 at 8 weeks after the anticipated birth date when puerperium is over, T3 at 14 months, and T4 at 2 years after the actual birth date. T3 and T4 go along with German parental allowance because T3 is at the time the parental allowance is over (if the couple took the full allowance and both parents were on parental leave for at least 2 months) and T4 is at the time the stretched out parental allowance (for families who

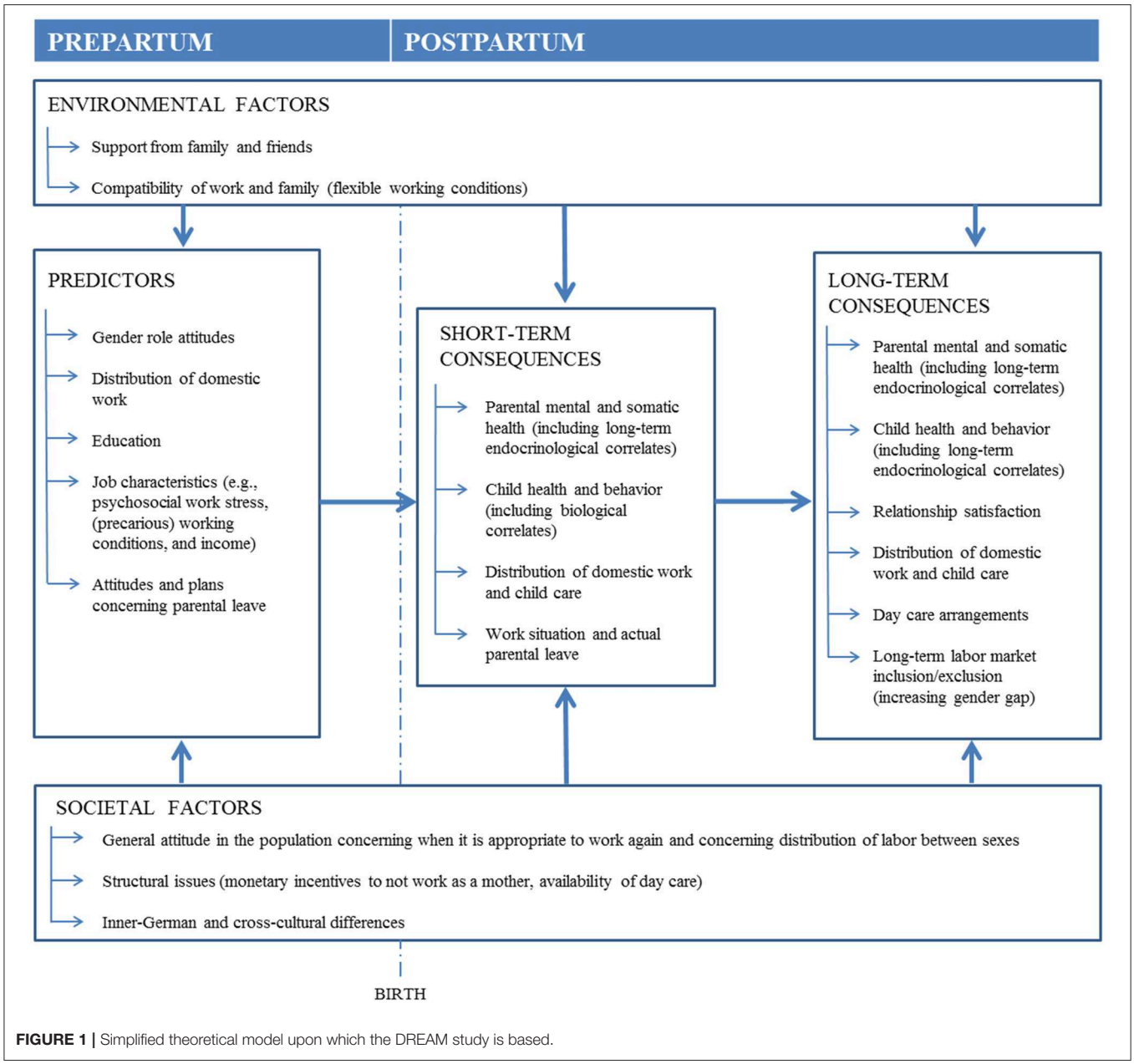

FIGURE 1 | Simplified theoretical model upon which the DREAM study is based.

worked part-time for a while) ends. A prolonged investigation of families into middle childhood is planned. The DREAM study is conducted in a larger city in East Germany where both maternal work participation and day care are a more frequent practice than in West Germany (Bundesministerium für Familie Senioren Frauen und Jugend, 2017a). Therefore, the DREAM study cooperates with a West German study called the Bremen Initiative to Foster Early Childhood Development ("BRISE"; Bremer Initiative zur Stärkung frühkindlicher Entwicklung). Moreover, data of the DREAM study will be compared to data of the Norwegian Akershus Birth Cohort (ABC study). This cross-cultural comparison to a country that is more progressive regarding gender considerations (Barker and Pawlak, 2011) will complement the results about the role of work participation for family health. For this purpose, measurement points and many instruments are matched.

Applying a multi-method approach, the DREAM study is complemented by two sub-studies: $DREAM_{HAIR}$ (analysis of HCC and other endogenous steroid hormones) and $DREAM_{TALK}$ (qualitative interviews regarding gender role values and distribution of domestic work, child care, and paid employment). Participants meeting inclusion criteria for the particular sub-study are contacted again to be invited to participate in the sub-study. Due to the focus of this paper, the basic DREAM study and the sub-study $DREAM_{HAIR}$ are presented in detail.

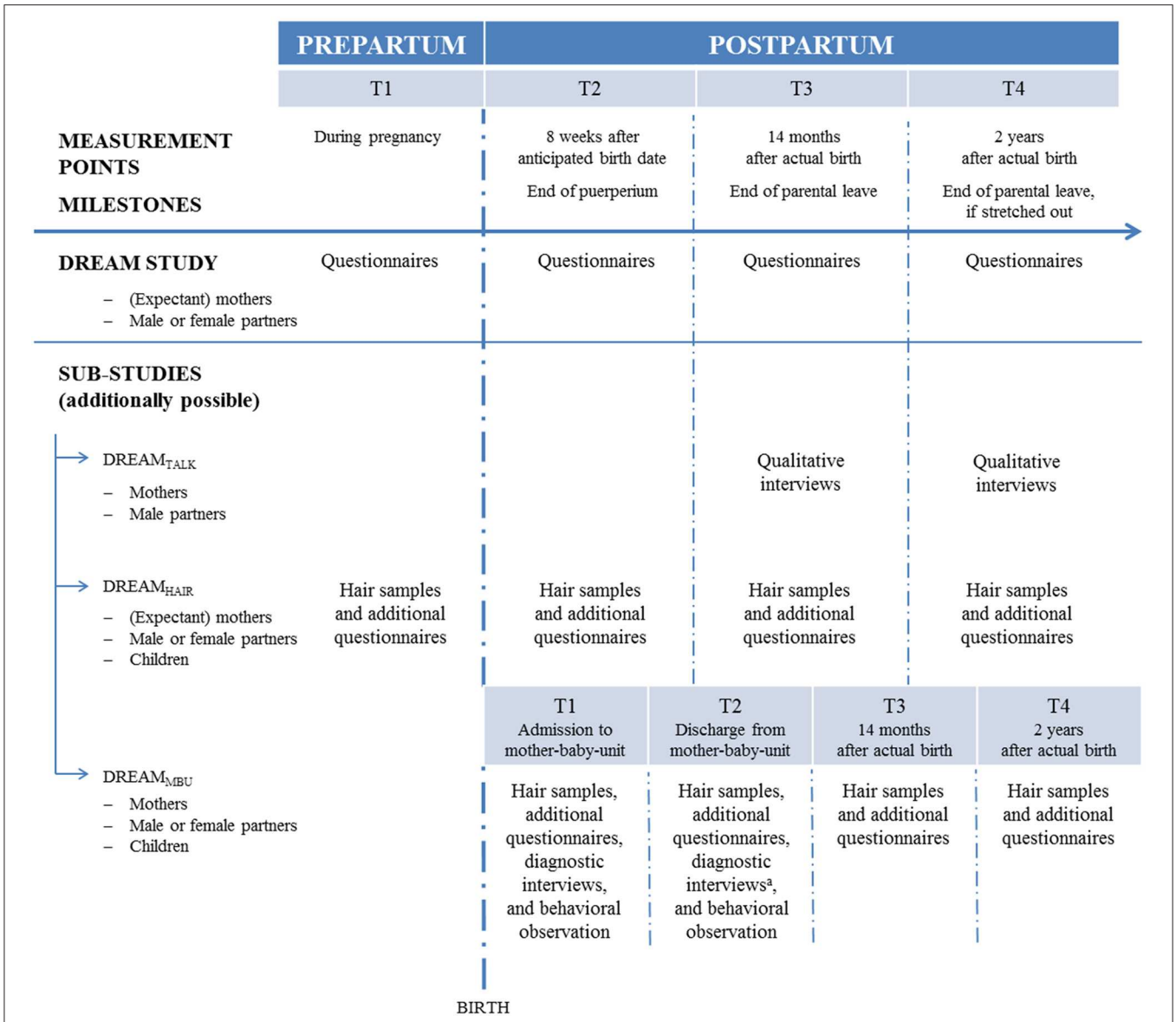

FIGURE 2 | Assessment waves of the DREAM study and its sub-studies $DREAM_{TALK}$ (qualitative interview sub-study), $DREAM_{HAIR}$ (long-term endocrinological sub-study), $DREAM_{MBU}$ (clinical sub-study). Regarding the community sample, T1 during pregnancy, T2 8 weeks after anticipated birth date, T3 14 months, and T4 2 years after actual birth date (prolongation into middle childhood planned). Regarding the clinical sample, T1 at admission and T2 at discharge (hair samples will be taken 2 months after discharge) from mother-baby-unit, T3 14 months (will only be omitted if the sampling point at discharge from mother-baby-unit is not too close, i.e., 14 months postpartum ± 2 months), T4 at 2 years after actual birth date. [a]At discharge, diagnostic interviews will only be omitted regarding children.

Sub-studies

$DREAM_{HAIR}$ and $DREAM_{MBU}$

The long-term endocrinological sub-study $DREAM_{HAIR}$ aims to examine the complex relationships between psychological distress and long-term steroid hormone levels in parent-child dyads from pregnancy to 2 years postpartum (prolongation into middle childhood planned). This sub-study consists of two branches: a community based approach ($DREAM_{HAIR}$, already started) and a clinical approach ($DREAM_{MBU}$, starting soon). Within the community branch, the community sample stems from the basic DREAM study. Within the clinical branch, a clinical sample of mothers with postpartum mental disorders, their partners (if available), and their children are recruited from a mother-baby day clinic. Data of the community and clinical sample will be combined for long-term analyses.

Recruitment for the community branch of the sub-study $DREAM_{HAIR}$ began 5 months after the start of the basic DREAM study. For this sub-study, all families whose expected delivery date is at least 4 weeks in the future are checked for inclusion criteria. Inclusion criteria for parents comprise a minimal hair length of 2 cm, no hair loss or baldness, no severe physical disease (e.g., cancer or adrenocortical dysfunction) over the last

5 years, and no use of glucocorticoid containing medication for the last 4 months. Regarding children, there are no particular reasons for exclusion other than no availability of hair (without a minimal hair length). Potentially confounding variables (e.g., medication, diseases) are assessed and used for further analyses. Inclusion criteria will be checked at every measurement point. If participants are excluded at a single measurement point due to temporary reasons, e.g., medication or too short hair, or they miss an assessment wave due to organizational reasons, they will be asked again at the next measurement point. Participants can join this sub-study as a couple or alone as long as each parent who has child custody provides her/his consent that child hair is also allowed to be taken.

After being enrolled into the basic DREAM study, hair samples of the eligible expectant mothers, their partners (i.e., $N = 152$ participants, i.e., 88 families, at T1 by the end of September 2018) and their children, are examined for HCC and other endogenous steroid hormones at four measurement points from pregnancy up to 2 years postpartum (**Figure 2**; prolongation into middle childhood planned). Regarding the parents, $T1_{DREAMHAIR}$ starts 4 to 6 weeks prior to the anticipated birth date which reflects HCC integrated during late pregnancy. Regarding the children, $T1_{DREAMHAIR}$ hair samples are taken soon after birth to gain information on intrauterine glucocorticoid regulation (Hollanders et al., 2017). The other measurement points ($T2_{DREAMHAIR}$, $T3_{DREAMHAIR}$, and $T4_{DREAMHAIR}$) are equivalent to the basic DREAM study.

At the respective first measurement point, hair samples of community sample will be taken at their home or at the lab as preferred by the participants while hair samples of the clinical sample will be taken at the lab in any case. In both samples, the first hair sample is taken by trained staff. In order to self-administer hair samples at the follow-ups, participants get a video teaching how to take own and child hair samples themselves or with help of their partner or a friend. At the follow-ups, participants get a reminder when the next hair samples are due. As we are aware that this sub-study requires some more commitment by the participants compared to the basic questionnaire study, we put emphasis on personal contact to the participants. For instance, trained staff assists them with taking own or child hair sample if needed.

For the clinical branch ($DREAM_{MBU}$), recruitment of a clinical sample of approximately $N = 70$ mothers with postpartum mental disorders who are treated in the day-care MBU at the Institute and Outpatient Clinics of Psychotherapy and Psychosomatic Medicine at the Technische Universität Dresden is planned starting by the year 2019 ($DREAM_{MBU}$). In this MBU, mothers with severe postpartum mental disorders (e.g., depressive disorders, anxiety disorders, obsessive-compulsive disorders, and personality disorders) are treated together with their child (0–12 months old, mean child age at admission is 23.3 weeks ($SD = 13.1$) for averagely 8.5 weeks ($SD = 3.1$). Based on a treatment concept that is state of the art for postpartum mental disorders (Wortmann-Fleischer et al., 2012), patients get a treatment consisting of both mother focused (e.g., disorder specific psychotherapy, psychopharmacotherapy) and mother-infant-interaction focused components (e.g., mother group treatment, video-interaction-therapy, baby massage and handling, sensitivity training) conducted by a multi-professional team. Partners and other family members are involved in the treatment if possible.

For the clinical sub-study $DREAM_{MBU}$, patients will be asked for written informed consent to take part in the study within the first week after admission. Their children and (if available) partners will also be included. As shown in **Figure 2**, hair samples of all participants will be taken four times: once at admission ($T1_{DREAMMBU}$) to measure the steroid exposition during the 2 months prior to treatment and a second time ($T2_{DREAMMBU}$) 2 months after discharge from MBU to assess HCC during the 2 months after treatment. Further, to compare the data of the clinical sample to the data of the community sample at $T3_{DREAMHAIR}$ and $T4_{DREAMHAIR}$ (14 and 24 months postpartum), a third ($T3_{DREAMMBU}$), and fourth ($T4_{DREAMMBU}$) hair sample of the clinical population will be taken [if the sampling point at discharge ($T2_{DREAMMBU}$) is very close to $T3_{DREAMHAIR}$ (14 ± 2 months), one sampling point will be omitted].

In the clinical part of the sub-study $DREAM_{MBU}$, patients and their partners will also go through the routine assessment of the treatment effects (**Table 1**) using a comprehensive assessment battery at admission and discharge. At admission, patients will be interviewed using the Structured Clinical Interview for DSM-IV (SCID I and SCID II; Wittchen et al., 1997). Both at admission and discharge, the Diagnostic Interview for Mental Disorders in Babies showing good to very good inter-rater reliability (Baby-DIPS; Popp et al., 2016), a semi-standardized observation of maternal sensitivity toward their baby's signals (Galeris, 2016; validation pending), and additional questionnaires regarding the maternal psychopathology and parenting as well as infant temperament, regulation disorders, and behavior will be obtained. There is an overlap between the MBU assessment and the basic DREAM assessment regarding all relevant measures.

$DREAM_{TALK}$

The qualitative $DREAM_{TALK}$ sub-study will start during summer 2019. A subsample targeting approximately $N = 40$ heterosexual couples, i.e., women and men engaged in a long-term relationship and living in the same household, will be interviewed on their gender role values and attitudes as well as thoughts about distribution of domestic work, child care, and paid employment. These attitudes will be compared to the actual situation. Additionally, both partners' satisfaction regarding their relationship and role distribution as well as how they explain a potential equality gap will be assessed. Results will be analyzed in relation to health-related outcomes measured in the basic DREAM study.

Based on a synthesis of previous studies on different task distributions (Crouter and Manke, 1997; Hall and MacDermid, 2009; Farrokhzad et al., 2010; Helms et al., 2010; Masterson and Hoobler, 2015; Bundesministerium für Familie Senioren Frauen und Jugend, 2017b), couples will be categorized into one of four groups by a cut-off threshold of hours spent on domestic

TABLE 1 | Constructs and instruments in the basic DREAM study.

Constructs	Instruments	Community sample (DREAM)				Clinical sample (DREAM$_{MBU}$)			
		T1	T2	T3	T4	T1	T2	T3	T4
SOCIODEMOGRAPHIC AND SOCIOECONOMIC FACTORS									
Nationality and mother tongue	Questions derived from the German National Cohort (German National Cohort Consortium, 2014)	x				x			
Education	Questions derived from the German National Cohort (German National Cohort Consortium, 2014)	x				x			
Marital status	Questions derived from the Socio-Economic Panel (SOEP; TNS Infratest Sozialforschung, 2016) and self-generated questions	x	x	x	x	x	x	x	x
Children and former pregnancies	Questions derived from the BRISE study based on the BabyCare Project (Friese and Kirschner, 2003)	x	x			x	x		
Housing	Questions derived from the German National Cohort (German National Cohort Consortium, 2014)	x	x	x	x	x	x	x	x
WORK-RELATED FACTORS									
Working hours and professional group	Questions derived from the German National Cohort (German National Cohort Consortium, 2014)	x		x	x	x	x	x	x
Job satisfaction and job burden	Questions derived from the BRISE study based on the BabyCare Project (Friese and Kirschner, 2003) and the Exploration Questionnaire for Identification of Differential Learning Paths in the Social Development in Toddler Age ("Explorationsfragebogen zur Identifikation differentieller Lernwege in den ersten beiden Lebensjahren", IDL 0-2; Petermann et al., 2002)	x		x	x	x	x	x	x
Sick leave and adaption of work situation in pregnancy	Questions derived from the ABC study (e.g., Dørheim et al., 2013)	x		x	x	x	x	x	x
Shift work	Questions derived from the BRISE study based on the BabyCare Project (Friese and Kirschner, 2003)	x		x	x				
Precariousness	Employment Precariousness Scale–Revised (EPRES; Vives et al., 2015)	x		x	x				
Psychosocial work stress	Effort-Reward Imbalance Questionnaire (ERI; Siegrist, 1996; Rödel et al., 2004)	x		x	x				
Work-privacy conflict	One scale (work-privacy conflict) of the Copenhagen Psychosocial Questionnaire (COPSOQ; Kristensen et al., 2005; Nübling et al., 2005)	x		x	x				
Plans and actual parental leave	Self-generated questions	x	x	x	x	x	x	x	x
Satisfaction with distribution of parental leave	Self-generated questions			x	x			x	x
DISTRIBUTION OF DOMESTIC WORK AND CHILD CARE									
Attitudes toward distribution of domestic work	Nine scales (effective communication about domestic labor, ministering to family needs, support of wage work, responsive to personal needs, avoiding conflict, coprovider orientation, valuing homemaking, standards, women's ultimate accountability) of the Orientation Toward Domestic Labor Questionnaire (ODL-Q; Hawkins et al., 1998)	x		x	x				
Distribution of domestic work and child care	Questions derived from the ABC study	x	x	x	x				
Time spent for domestic work and child care	Questions derived from the 1997 National Study of the Changing Workforce (Hall and MacDermid, 2009)			x	x				
SOMATIC FACTORS									
Current and former somatic health	Questions derived from the ABC study (e.g., Garthus-Niegel et al., 2018; Junge et al., 2018)	x	x	x	x	x	x	x	x
Exercise and physical activity	Questions derived from the ABC study generated by health professionals (e.g., Gjestland et al., 2013)	x		x	x	x	x	x	x
Health-related quality of life	Short-Form Health Survey (SF-8; Ware et al., 2001; Ellert et al., 2005)				x				x

(Continued)

TABLE 1 | Continued

Constructs	Instruments	Community sample (DREAM)				Clinical sample (DREAM$_{MBU}$)			
		T1	T2	T3	T4	T1	T2	T3	T4
Drugs	Questions derived from the ABC study generated by health professionals (e.g., Nordeng et al., 2012)	x		x	x	x	x	x	x
Smoking	Slightly modified questions derived from the German National Cohort (German National Cohort Consortium, 2014)	x	x	x	x	x	x	x	x
Alcohol	Questions derived from the German National Cohort (German National Cohort Consortium, 2014)	x	x	x	x	x	x	x	x
MENTAL FACTORS									
Current and former mental disorders and treatments	Self-generated questions Structured Clinical Interview for DSM-IV (SCID I and SCID II; Wittchen et al., 1997)		x	x	x	x x	x	x	x
Use of early help	Self-generated questions		x			x			
Symptoms of depression	Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987; Bergant et al., 1998)	x	x	x	x	x	x	x	x
Psychopathological symptoms	Four scales (somatization, obsessiveness, anxiety and anger/hostility) of the Symptom Check List–Revised (Derogatis, 1977; Franke and Derogatis, 2002) Brief symptom inventory (BSI; Derogatis, 1993; Franke, 2000)	x	x	x	x	x	x	x	x
Current and former critical life events	Questions derived from the BRISE study based on the Avon Longitudinal Study of Parents and Children (ALSPAC; Thomson et al., 2014)		x	x		x	x	x	
Current and former posttraumatic stress reactions	Posttraumatic Diagnostic Scale (PDS; Ehlers et al., 1996; Foa et al., 1997)	x[c]		x[c]	x[c]	x	x	x	x
Adverse childhood experiences	Childhood Trauma Questionnaire (CTQ; Bernstein and Fink, 1998; Wingenfeld et al., 2010)	x[c]				x			
Intradyadic stress and extradyadic stress from daily hassles	Multidimensional Stress Questionnaire for Couples ("Multidimensionaler Stressfragebogen für Paare", MDSP; Bodenmann, 2007)				x				x
RELATIONSHIP FACTORS									
Relationship satisfaction	Short version of the Partnership Questionnaire ("Kurzform des Partnerschaftsfragebogens," PFB-K; Kliem et al., 2012)	x	x	x	x	x	x	x	x
Social support	Short version of the Social Support Questionnaire ("Fragebogen zur Sozialen Unterstützung," F-SozU-14; Fydrich et al., 2009)	x		x	x	x	x	x	x
PREGNANCY AND BIRTH-RELATED FACTORS									
Fear of childbirth[a]	Fear of Birth Scale (FOBS; Haines et al., 2011)	x							
General information and complications during pregnancy and birth[a]	Maternity records ("Mutterpass"; Gemeinsamer Bundesausschuss, 2015), questions derived from the BRISE study, and self-generated questions		x			x			
Birth experience[b]	Salmon's Item List (SIL; Stadlmayr et al., 2001)		x			x			
Overall birth experience[b]	Question derived from the ABC study (e.g., Garthus-Niegel et al., 2014)		x			x			
Fear for oneself/mother and child during birth[b]	Self-generated questions based on the ABC study (e.g., Garthus-Niegel et al., 2013)		x			x			
Fear of a prospective birth[b]	Question derived from the ABC study		x			x			
Birth-related post-traumatic stress reactions	Impact of Event Scale–Revised (IES-R; Weiss and Marmar, 1996; Maercker and Schützwohl, 1998)		x			x			

(Continued)

TABLE 1 | Continued

Constructs	Instruments	Community sample (DREAM)				Clinical sample ($DREAM_{MBU}$)			
		T1	T2	T3	T4	T1	T2	T3	T4
CHILD-RELATED FACTORS									
Breastfeeding	Self-generated questions based on recommendations of the World Health Organization (World Health Organization, 2009)		x	x	x	x	x	x	x
Parent-to-infant-bonding	Postpartum Bonding Questionnaire (PBQ; Brockington et al., 2001; Reck et al., 2006)		x	x	x	x	x	x	x
Sensitivity toward baby's signals	Semi-standardized observation of maternal sensitivity (global score and six subscales) toward baby's signals (Galeris, 2016)					x	x		
Parenting sense of competence	Parenting Sense of Competence Scale ("Fragebogen zum Kompetenzgefühl von Eltern," FKE; Gibaud-Wattston and Wandersman, 1978, as cited in Johnston and Mash, 1989; Miller, 2001)					x	x	x	x
Parenting stress	Parenting Stress Index ("Eltern-Belastungs-Inventar," EBI; Abidin, 1995; Tröster, 2011)					x	x	x	x
Infant regulation disorders	Structured Diagnostic Interview for Regulatory Problems in Infancy ("Diagnostisches Interview bei psychischen Störungen im Säuglings- und Kleinkindalter," Baby-DIPS; Popp et al., 2016)					x	x		
Child health	Medical records ("Kinderuntersuchungsheft"; Gemeinsamer Bundesausschuss, 2016) and questions derived from the ABC study		x	x	x	x	x	x	x
Child development	Five scales (communication, gross motor, fine motor, problem solving, personal-social) of the 14 and 24 month version of the Ages and Stages Questionnaire-3 (ASQ-3; Squires and Bricker, 2009)			x	x			x	x
Child temperament	One scale (fussy/difficult scale) of the Infant Characteristics Questionnaire (ICQ; Bates et al., 1979)		x						
	Infant Behavior Questionnaire (IBQ; Rothbart, 1981; Pauli-Pott et al., 1999)					x			
Child care	Questions derived from the Socio-Economic Panel (SOEP; TNS Infratest Sozialforschung, 2016)			x	x	x	x	x	x
PERSONALITY	Big Five Inventory–SOEP (BFI-S; Schupp and Gerlitz, 2008)		x						
METACOGNITION	Metacognition Questionnaire–Short version (MFK-30; Wells and Cartwright-Hatton, 2004; Arndt et al., 2011)		x						

Regarding community sample (DREAM), T1 during pregnancy, T2 8 weeks after anticipated birth date, T3 14 months, and T4 2 years after actual birth date (prolongation into middle childhood planned). Regarding clinical sample ($DREAM_{MBU}$), T1 at admission and T2 at discharge (hair samples will be taken 2 months after discharge) from mother-baby-unit, T3 14 months (will only be omitted if the sampling point at discharge from mother-baby-unit is not too close, i.e., 14 months postpartum ± 2 months), T4 at 2 years after actual birth date. [a]*Only for expectant mothers.* [b]*Male and female partners only asked when they have attended birth.* [c]*Only for* $DREAM_{HAIR}$.

work, child care, and paid employment to compare those groups: progressive couples sharing domestic work, child care, and paid work equally; traditional couples with women doing most of the domestic work and child care and men doing most of the paid work; as well as two interjacent groups.

As shown in **Figure 2**, qualitative interviews, specifically problem-centered interviews (PCI) following Witzel and Reiter (2012), will be carried out a few weeks after T3 and T4 of the basic DREAM study. This is at the time the regular and the stretched out parental allowance run out. The interviews will be analyzed using qualitative content analysis by Mayring (2010). Categories for the collected interview data will be established, yielding interpretations of the participants' answers in a replicable and systematic way.

Taking into account the participants' wishes, the interviews will take place at the couples' home or at the lab. Trained doctoral students following interview guidelines will conduct them. The project team aims to achieve a personal connection and identification with the study among the participants in order to keep attrition low.

Materials

The DREAM study is characterized by a multi-method approach combining quantitative questionnaires (basic DREAM study), long-term endocrine correlates ($DREAM_{HAIR}$), and qualitative interviews ($DREAM_{TALK}$). Study data are collected and managed using Research Electronic Data Capture (REDCap), a secure, web-based application designed to support data capture for research studies, hosted at the "Koordinierungszentrum für Klinische Studien" at the Faculty of Medicine of the Technische Universität Dresden, Germany (Harris et al., 2009). In this article, we focus on the questionnaire data and analysis of HCC.

Questionnaires

The DREAM study comprises several questionnaires as presented in **Table 1**. Standardized and validated instruments with good psychometric properties were preferably used. For example, the German version of the Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987; Bergant et al., 1998), a well-established, reliable, and valid instrument was obtained to measure prepartum and postpartum symptoms of depression, while the Effort-Reward Imbalance Questionnaire (ERI; Siegrist, 1996; Rödel et al., 2004) being characterized with satisfactory psychometric characteristics was used to assess psychosocial work stress. Where possible, instruments were chosen in agreement with the BRISE project team, the German National Cohort (German National Cohort Consortium, 2014), or the Norwegian ABC study to allow intra- and intercultural comparisons. If no German version of a questionnaire existed, the English or Norwegian version was translated into German and back-translated by a native speaker (validation still pending).

In the sub-studies, relevant questionnaires were added. In $DREAM_{HAIR}$, participants complete a self-generated questionnaire about hair-related characteristics, e.g., washes per week, curls, or hair treatments (as described in Stalder et al., 2014). Additionally, hair analyses were complemented by trauma specific questionnaires (**Table 1**). Specifically, the checklist of the Posttraumatic Diagnostic Scale (PDS; Ehlers et al., 1996; Foa et al., 1997) to assess the nature and presence of the most upsetting traumatic event (A1 and A2 criteria of DSM-IV, time since occurrence) as well as the Childhood Trauma Questionnaire (CTQ; Bernstein and Fink, 1998; Wingenfeld et al., 2010) to measure the severity of childhood maltreatment were added. The German adaptions of both questionnaires have been found to be reliable and valid instruments similar to their English original versions (Griesel et al., 2006; Wingenfeld et al., 2010).

In order to attain best possible response and retention rates, participants can decide whether to complete online or paper-pencil versions of the questionnaires. By the end of September 2018, $N = 1{,}410$ participants, i.e., expectant mothers and their partners, have given consent to join the study and completed the first questionnaire which is necessary to take part in the follow-ups. Almost half of them (47.6%; $n = 671$) decided for the paper-pencil procedure with a somewhat larger ratio of expectant mothers (50.7%; $n = 408$) compared to their partners (43.5%; $n = 259$). After completing T1, the participants receive the subsequent questionnaires and reminders in the desired manner automatically, even if they missed one of the measurement points. To keep attrition low, participants get incentives together with the follow-up questionnaires at every measurement point, e.g., rompers, bibs, or books, to encourage them to answer in time. In case of breakup, participants will be contacted separately, so this is no reason to drop out.

Hair Cortisol Concentrations (HCC) Analyses

For analyses of HCC as part of the sub-study $DREAM_{HAIR}$, a hair strand of a diameter of ∼3 mm is taken scalp-near from a posterior vertex position. HCC will be determined in the 2 cm hair segment most proximal to the scalp. If permitted by the individuals' hair length, the next 2 cm of hair will additionally be analyzed. Based on a hair growth rate of ∼1 cm/month (Wennig, 2000), these hair segments are assumed to represent integrated, cumulated cortisol levels over the 2 month or, if permitted, 4 month period prior to hair sampling. Hair samples will be stored in aluminum foil and sent to the Institute of Biological Psychology at the Faculty of Psychology of the Technische Universität Dresden. Hair analyses will be conducted following the liquid chromatography-tandem mass spectrometry (LC-MS/MS) protocol which is characterized by very good sensitivity, specificity, and reliability (Gao et al., 2013). Besides cortisol, the LC-MS/MS protocol allows the quantitative analysis of other endogenous steroid hormones, i.e., cortisone, testosterone, progesterone, corticosterone, dehydroepiandrosterone (DHEA), and androstenedione that may serve as important mediators in the development of psychopathology or psychological distress (Gao et al., 2013).

Sample

In this section, the interim samples of the basic DREAM study and the sub-study $DREAM_{HAIR}$ (community branch) by the end of September 2018 will be presented. The participants get no payment for their participation but incentives at every measurement point, e.g., rompers or bibs, as mentioned earlier. Additionally, a lottery is established so two participants may win vouchers and gifts every month.

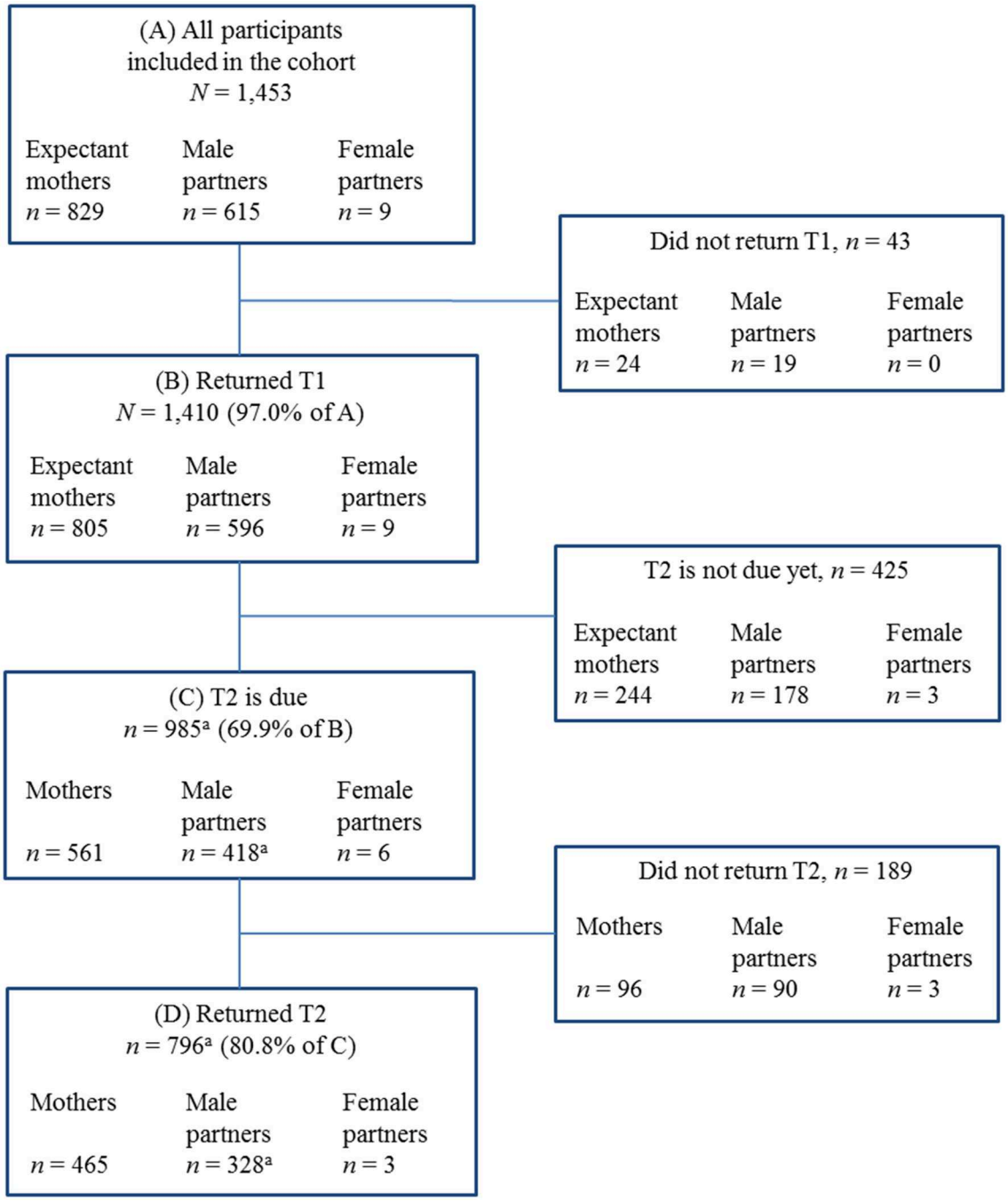

FIGURE 3 | Flow chart of the DREAM study. Notes: T1 during pregnancy, T2 8 weeks after anticipated birth date. Data from end of September 2018 (data collection is not finished yet, recruitment ongoing). Further future assessment waves (not due yet): T3 14 months and T4 2 years after actual birth date (prolongation into middle childhood planned). [a]Male partners' sample includes one male partner who did not return T1 but returned T2.

Interim Sample and Flow Chart of DREAM

The DREAM study addresses expectant mothers and their male or female partners in or around Dresden, Germany. The flow chart of the DREAM study is presented in **Figure 3**.

$N = 1{,}453$ expectant mothers and their partners have provided their written consent to participate from June 2017 to the end of September 2018 (recruitment still ongoing). Of those having provided consent, the vast majority of $N = 1{,}410$ (97.0%) participants completed the first questionnaire (T1) resulting in a sample of $n = 805$ expectant mothers (57.1%) and $n = 596$ male partners (42.3%), plus $n = 9$ female partners (0.6%). At T1, most of the participants joined the study as a couple (81.3%;

TABLE 2 | Total sample who returned T1 ($N = 1,410$) according to recruitment way.

Recruitment way	Sample ($N = 1,410$)	
	n	*(%)*
Obstetrical clinics ($N = 8$): information evenings and tours of the delivery rooms	1,049	(74.4)
- *there of Dresden* ($N = 5$)	961	(91.6)
- *there of surrounding area* ($N = 3$)	88	(8.4)
Obstetrical clinic ($N = 1$): scheduled birth registration	38	(2.7)
Obstetrical clinic ($N = 1$): inpatient prenatal treatment ward	33	(2.4)
Freestanding birthing center ($N = 1$): information evenings	58	(4.1)
Midwife practices ($N = 12$): birth preparation courses	95	(6.7)
City of Dresden: early care ("Frühe Hilfen")	2	(0.1)
Other places	62	(4.4)
Unknown places	73	(5.2)

$n = 1,146$ people, i.e., 573 couples), but in some cases an expectant mother participated without a partner (16.4%; $n = 232$) and a few partners participated without the expectant mother (2.3%; $n = 32$).

As shown in **Table 2**, participants who returned T1 were recruited in obstetrical clinics in Dresden and the surrounding area (79.5%; $n = 1,120$), i.e., at information events and tours of the delivery rooms, scheduled birth registration done by midwives, and at the inpatient prenatal treatment ward. Further participants were included from a freestanding birthing center (4.1%; $n = 58$), at birth preparation courses in midwife practices (6.7%; $n = 95$), and at the early care of the city of Dresden ("Frühe Hilfen"; 0.1%; $n = 2$). A certain number of participants (4.4%; $n = 62$) were recruited via other places, i.e., mainly via announcements in gynecological outpatient settings, child-related stores, websites, newspapers, and magazines of health insurances, or through personal contact. Also, some participants (5.2%; $n = 73$) could not be assigned to a specific recruitment way.

The number of different people approached was varying between the locations. For example, 25–100% of the attendees of the birth preparation courses knew the study already as they often visited at least one of the clinics' information evenings as well. In sum, there was personal contact to $N = 9,477$ persons (i.e., expectant mothers and their male or female partners). Given that $n = 1,348$ participants provided written informed consent (not including those who were recruited via other places) this would indicate a response rate of 14.2%. However, the true response rate is considerably higher but hard to calculate exactly because a much smaller number was eligible due to multiple visits.

By the end of September 2018, 80.8% ($n = 796$) of those participants T2 was due for ($n = 985$) have sent back the second questionnaire which is 8 weeks after the expected delivery date. The retention rate from T1 to T2 is best for mothers (82.9%; $n = 465$) followed by male (78.5%; $n = 328$) and female partners (50.0%; $n = 3$). As some participants do not send back the follow-ups in time but with some delay, we expect the retention rate to increase in the further course of the study.

Interim Sample and Flow Chart of $DREAM_{HAIR}$

$N = 603$ participants, i.e., 317 families, were eligible for $DREAM_{HAIR}$ as the anticipated delivery date was at least 4 weeks in the future (for flow chart of $DREAM_{HAIR}$ see **Supplementary Figure 1**). Of those, $n = 58$ participants have been excluded at T1 due to drug intake, medical conditions, hair characteristics, or organizational reasons. These participants might be included again at future measurement points. Further $n = 257$ participants could not be enrolled because of lack of response or interest. For $n = 136$ participants T1 had not been due by the end of September 2018. As a result, the sample of $DREAM_{HAIR}$ has consisted of $N = 152$ participants, i.e., 88 families, at this time. This is $n = 87$ expectant mothers, $n = 64$ male partners, and $n = 1$ female partner who returned hair samples at T1. As the T1 sampling of the child is due a few days after childbirth, $n = 59$ child samples (with one pair of twins) have been taken at T1 so far. In some cases, child hair was too short to be taken ($n = 13$). Further $n = 17$ child hair samples were not due yet.

T2 was due for $n = 98$ participants, i.e., 58 families from whom the majority (87.8%) returned T2 in time. Regarding child hair at T2, $n = 47$ samples out of $n = 59$ children (with one pair of twins) were returned. Further $n = 6$ child samples were not available because hair was too short to be taken and $n = 6$ samples were not returned in time. In addition, $n = 9$ participants, i.e., 5 families (with $n = 5$ children) joined the $DREAM_{HAIR}$ study initially at T2 due to organizational reasons.

Planned Data Analyses

The DREAM study provides quantitative data of four measurement points during the course of pregnancy to (at least) 2 years postpartum. Therefore, data will be analyzed both cross-sectionally and longitudinally with regard to the respective objectives of the study. More specifically, for the longitudinal analyses, multiple linear regression analyses, simple logistic regression analyses, and multinomial logistic regression analyses will be conducted to examine how prepartum individual and family factors predict outcomes after birth, such as health of family members and mothers' and their partners' participation in the labor market. As we are dealing with panel data, we will also perform fixed effects regression analyses in order to control for unobserved heterogeneity. Moreover, latent growth curve analyses in the framework of structural equation modeling (Bollen and Curran, 2006) and autoregressive cross-lagged analyses will be conducted when testing whether prepartum factors predict changes in social, work, and stress factors after birth. Full information maximum likelihood estimation or multiple imputation techniques will be used, as such techniques are considered to the most adequate approaches to handle missing data (Schafer and Graham, 2002).

We will analyze data on the individual level, while we will also have the opportunity to control for the partners' reports when

both mother and partner participated as a couple. If both mother and her partner (and their baby, with respect to $DREAM_{HAIR}$ and $DREAM_{MBU}$) participate in the study, data sets can be combined using a family code.

Then, multi-level modeling as a beneficial statistical approach exceeding conventional methods will be applied, enabling us to analyze relations within and between dyads or triads while taking into account the shared variance in dyadic (couples) or triadic (couples and their child) structure of hierarchical data (Woltman et al., 2012; Davis et al., 2018). Multi-level modeling is also well-suited for examining changes over an extended period of time with several measurement points without being limited by missing data (Woltman et al., 2012).

Power Analyses

Power analyses for the basic DREAM study and $DREAM_{HAIR}$ were conducted by means of Monte Carlo simulations using the R package "semsim," version 0.5–13 (Beaujean, 2014). We conducted Monte Carlo simulation studies with 10,000 samples and examined the stability of our results by re-running the analyses with three different seeds. Power analyses were performed assuming a multivariate normal distribution of all variables. Moreover, we also estimated power with multivariate distributions deviating from normality. More specifically, in accordance with the methodological literature (Muthén and Asparouhov, 2002), we examined power for non-normal distributions with a skewness of 2.0 and a kurtosis of 3.5. Non-normal distributions were generated by using the multivariate Fleishman transformation (Vale and Maurelli, 1983). We also varied the proportion of missingness in the dependent variable (which is assumed to be measured at T4 where attrition is an important issue) from 0 to 70%.

First, we estimated power when the whole DREAM targeted sample of 2,000 couples, i.e., 4,000 individuals, are used in the analyses. With a sample size of $N = 2{,}000$, power analyses for multiple regression analyses with a continuous construct such as depressive symptoms as outcome variable (one of the main outcome variables in the study) and five continuous predictors showed a very high statistical power of 99% or above to detect significant effects given a small effect size of $\beta = 0.20$ between predictors and the outcome and a 5% (two-sided) type I error. Such high power was obtained even for non-normally distributed data and up to 70% proportion of missing data in the outcome variable. Therefore, the sample size at T4 will be large enough to obtain very high statistical power even with a dropout rate of 70% over the four assessment points.

As the number of same-sex couples in our study is very small, our statistical power will most likely not be sufficient to perform subgroup analyses. Therefore, we would consider these family constellations as case studies, which can be used to generate hypotheses for further studies with a targeted focus on families with same-sex parents.

Regarding the sub-study $DREAM_{HAIR}$, approximately 20% of participants of the basic DREAM study participate in $DREAM_{HAIR}$; hence we estimate $N = 360$ couples, i.e., 720 individuals, will participate at T1. For $N = 360$, even with a dropout rate of 40% at T4, Monto Carlo simulations estimated a power of 93% (multivariate normal distribution) and 80% (non-normally distributed data) to detect small effect sizes of $\beta = 0.20$ between predictors and the outcome in multiple regression analyses with five predictors and a 5% (two-sided) type I error. Moreover, a somewhat larger effect size of $\beta = 0.28$ will be detected with a power of 94% (multivariate normal distribution) and a power of 80% (non-normally distributed data) in the unlikely case that attrition will be as high as 70%.

With respect to the sub-study $DREAM_{MBU}$, the clinical sample will be much smaller ($N = 70$ mothers with their babies and, if available, partners). We expect a higher compliance of the clinical branch (compared to the community branch), as women are personally bound to our team. Specifically, this sample size allows to detect medium effects at an α-level of 0.05 and a statistical power of 0.80 (repeated measures ANOVA, four measurement points), assuming a moderate correlation ($r = 0.50$) among repeated measures as conducted using G*Power 3.1 (Faul et al., 2007, 2009). As we will be able to recruit participants for the clinical sample for a longer period than for the community sample, our sample size then will be sufficient to allow subgroup analyses as a function of responder status or pre-treatment HCC.

Regarding $DREAM_{TALK}$, we are targeting $N = 40$ couples. In qualitative research, the sample size can generally be determined by saturation. While the sample size should be large enough to sufficiently describe the phenomenon of interest (Glaser and Strauss, 1967; Malterud et al., 2016), saturation is reached when no further information is gained by interviewing further participants (Kvale, 1996). Different authors have suggested sample sizes between 20 and 50 (Morse, 1994; Creswell and Poth, 2013). Therefore, we are confident that our subsample size will be large enough to reach saturation.

Altogether, the targeted sample size of the basic DREAM study and the subsample sizes of the sub-studies will be large enough to answer the main research questions.

FIRST RESULTS

In this chapter, preliminary results regarding the sample of the basic DREAM study are presented.

Sociodemographic Characteristics

Tables 3, **4** show preliminary sociodemographic characteristics of the basic DREAM study sample during pregnancy (T1). The majority of expectant women (96.0%), male partners (95.6%), and all female partners reported German to be the mother tongue. On average, at T1 expectant mothers were in gestational week 29.9 ($SD = 6.1$; $Range = 10$–41) and most had a singleton pregnancy (98.0%) while $n = 11$ women were pregnant with twins and $n = 2$ women with multiples. For the majority of them, it was the first child (expectant mothers: 78.3%; male partners: 76.0%; female partners: 77.8%).

Mean age of expectant mothers was 30.1 years ($SD = 4.0$; $Range = 15$–42), 32.4 years of male partners ($SD = 5.0$; $Range = 22$–56), and 35.4 years of female partners ($SD = 6.5$; $Range = 28$–47).

TABLE 3 | Sociodemographic characteristics of expectant mothers and their partners during pregnancy (T1).

	Expectant mothers (*n* = 805)	**Male partners to the mother (*n* = 596)**	**Female partners to the mother (*n* = 9)**
Age	30.1 ± 4.0 (15-42)	32.4 ± 5.0 (22-56)	35.4 ± 6.5 (28-47)
Marital status			
Married/registered same sex partnership	336 (41.7)	259 (43.4)	8 (88.9)
Unmarried	441 (54.8)	311 (52.2)	1 (11.1)
Divorced	25 (3.1)	22 (3.7)	0 (0.0)
Widowed	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	2 (0.3)	0 (0.0)	0 (0.0)
Missing data	1 (0.1)	4 (0.7)	0 (0.0)
Partnership			
Yes	791 (98.3)	588 (98.7)	9 (100.0)
Living together			
Yes, permanently	745 (94.2)	555 (94.4)	9 (100.0)
Yes, not permanently	35 (4.4)	26 (4.4)	0 (0.0)
No	7 (0.9)	5 (0.9)	0 (0.0)
Missing data	4 (0.5)	2 (0.3)	0 (0.0)
No	9 (1.1)	2 (0.3)	0 (0.0)
Missing data	5 (0.6)	6 (1.0)	0 (0.0)
Number of children			
0	630 (78.3)	453 (76.0)	7 (77.8)
1	143 (17.8)	101 (17.0)	1 (11.1)
2	20 (2.5)	24 (4.0)	0 (0.0)
3	4 (0.5)	4 (0.7)	0 (0.0)
4	2 (0.2)	0 (0.0)	0 (0.0)
Missing data	6 (0.7)	14 (2.3)	1 (11.1)
Education			
No degree	0 (0.0)	2 (0.3)	0 (0.0)
Lower secondary education level 2	7 (0.9)	16 (2.7)	0 (0.0)
Secondary school certificate	164 (20.4)	157 (26.3)	5 (55.6)
Advanced technical college entrance qualification	66 (8.2)	51 (8.6)	2 (22.2)
A-level through second chance education	17 (2.1)	16 (2.7)	0 (0.0)
Subject-related or higher education entrance qualification (A-level)	548 (68.1)	341 (57.2)	2 (22.2)
Still in school	2 (0.2)	0 (0.0)	0 (0.0)
Unknown	1 (0.1)	2 (0.3)	0 (0.0)
Missing data	0 (0.0)	11 (1.9)	0 (0.0)
Professional qualification			
No qualification	4 (0.5)	4 (0.7)	0 (0.0)
Occupational apprenticeship	285 (35.4)	198 (33.2)	6 (66.7)
Master of crafts	30 (3.7)	47 (7.9)	1 (11.1)
University	434 (53.9)	277 (46.4)	2 (22.2)
Doctoral degree	34 (4.2)	32 (5.4)	0 (0.0)
Still in qualification	15 (1.9)	20 (3.4)	0 (0.0)
Missing data	3 (0.4)	18 (3.0)	0 (0.0)
Working hours per week	37.5 ± 8.9 (2-68)	40.9 ± 7.7 (1-70)	40.7 ± 3.7 (35-45)
Net earnings on average			
Up to 450 €	25 (3.1)	14 (2.3)	0 (0.0)
451 € to 850 €	25 (3.1)	11 (1.9)	0 (0.0)
851 € to 1,500 €	224 (27.8)	106 (17.8)	4 (44.4)
1,501 € to 2,500 €	417 (51.8)	307 (51.5)	5 (55.6)
More than 2,500 €	73 (9.1)	125 (21.0)	0 (0.0)
Not working or missing data	41 (5.1)	33 (5.5)	0 (0.0)

(Continued)

TABLE 3 | Continued

	Expectant mothers ($n = 805$)	Male partners to the mother ($n = 596$)	Female partners to the mother ($n = 9$)
Job burden			
Not burdened	73 (9.1)	54 (9.1)	0 (0.0)
A little bit burdened	254 (31.5)	163 (27.3)	2 (22.2)
Moderately burdened	269 (33.4)	254 (42.6)	3 (33.4)
Heavily burdened	100 (12.4)	79 (13.3)	2 (22.2)
Very heavily burdened	7 (0.9)	11 (1.8)	1 (11.1)
Not working or missing data	102 (12.7)	35 (5.9)	1 (11.1)
Job satisfaction			
Very satisfied	103 (12.8)	94 (15.8)	1 (11.1)
Quite satisfied	323 (40.1)	282 (47.3)	4 (44.5)
Neither unsatisfied nor satisfied	132 (16.4)	111 (18.6)	2 (22.2)
Quite unsatisfied	94 (11.7)	57 (9.6)	1 (11.1)
Very unsatisfied	48 (6.0)	18 (3.0)	1 (11.1)
Not working or missing data	105 (13.0)	34 (5.7)	0 (0.0)
Intention to take parental leave			
Yes	757 (94.0)	477 (80.0)	7 (77.8)
No	5 (0.6)	61 (10.3)	2 (22.2)
Unknown	2 (0.3)	27 (4.5)	0 (0.0)
Missing data	41 (5.1)	31 (5.2)	0 (0.0)
Duration of parental leave as intended	14.4 ± 5.0 (3-36)	3.3 ± 3.0 (1-24)	4.4 ± 3.7 (2-12)

n (%) or M ± SD (Range).

Compared to the overall German population (Statistisches Bundesamt, 2018a,b) and the population of Dresden (Statistisches Landesamt Sachsen, 2018), participants are characterized by a rather high educational and professional level and full-time work status.

Regarding employment 1 year ago, i.e., prior to pregnancy, first analyses showed that a greater ratio of male partners (81.2%; $n = 484$) than expectant mothers (61.9%; $n = 498$) had a full-time position ($\chi^2 = 62.153$; $df = 1$; $p < 0.01$). Accordingly, a part-time position was hold by a greater ratio of expectant mothers (23.0%; $n = 185$) than male partners (6.9%; $n = 41$; $\chi^2 = 66.032$; $df = 1$; $p < 0.01$). These gender differences could be found irrespective of whether expecting the first child or already having children, i.e., the ratio holding a full-time position was lower in first-time mothers (72.1%; $n = 446$) compared to first-time fathers (80.4%; $n = 360$; $\chi^2 = 9.702$; $df = 1$; $p < 0.01$) as well as in mothers (28.4%; $n = 48$) compared to fathers already having children (87.4%; $n = 111$; $\chi^2 = 101.521$; $df = 1$; $p < 0.01$). Likewise, the ratio working part-time was higher both in first-time mothers and mothers already having children compared to the respective men.

In fact, as shown in **Table 5**, these gender differences regarding employment 1 year ago, i.e., prior to pregnancy, were more pronounced in parents who already had children compared to first-time parents. Hence, among expectant mothers, first-time mothers (72.1%; $n = 446$) were working full-time more often than mothers already having children (28.4%; $n = 48$; $\chi^2 = 108.140$; $df = 1$; $p < 0.01$). Regarding part-time work, it was vice-versa, i.e., a higher ratio of mothers already having children (47.9%; $n = 81$) compared to first-time mothers (16.5%; $n = 102$) was working part-time ($\chi^2 = 73.648$; $df = 1$; $p < 0.01$). In male partners, no such a difference between first-time fathers and fathers already having children could be found regarding a full-time (80.4%; $n = 360$ vs. 87.4%; $n = 111$; $\chi^2 = 3.314$; $df = 1$; $p = 0.07$) or part-time position (7.1%; $n = 32$ vs. 7.1%; $n = 9$; ($\chi^2 = 0.000$; $df = 1$; $p = 0.98$).

Regarding current employment, i.e., during pregnancy, nearly one third of expectant mothers was in employment ban because of a health risk for mother or child (29.7%; $n = 239$). Further, the number of expectant mothers currently holding a full-time (44.6%; $n = 359$; $\chi^2 = 101.327$; $df = 1$; $p < 0.01$) and part-time position (16.8%; $n = 135$; $\chi^2 = 23.539$; $df = 1$; $p < 0.01$) was lower than 1 year ago (i.e., prior to pregnancy). In male partners, no such decrease regarding a full-time ($\chi^2 = 1.333$; $df = 1$; $p = 0.25$) and part-time position (exact $p = 0.63$) within the last year was found.

During pregnancy, the majority in all groups reported that they intended to take parental leave. Expectant mothers and male partners differed in the intention to take parental leave (94.0 vs. 80.0%; $\chi^2 = 105.16$, $df = 2$; $p < 0.01$). Further, expectant mothers intended to take parental leave for a longer period of time ($M = 14.4$ months; $SD = 5.0$; $Range = 3$–36) than male partners ($M = 3.3$ months; $SD = 3.0$; $Range = 1$–24; $t_{(1209.48)} = 48.57$, $p < 0.01$, *BCa 95%-CI* [10.63; 11.56]).

Birth-Related Characteristics

By the end of September 2018, birth-related characteristics at T2 (**Table 6**) were available for $n = 465$ mothers who had given birth

TABLE 4 | Former (1 year ago) and current employment during pregnancy (T1).

	Expectant mothers ($n = 805$) *n (%)*	Male partners to the mother ($n = 596$) *n (%)*	Female partners to the mother ($n = 9$) *n (%)*
Former employment (1 year ago)			
No employment	9 (1.1)	5 (0.8)	0 (0.0)
Not regularly employed	8 (1.0)	6 (1.0)	0 (0.0)
Marginal employment	39 (4.8)	23 (3.9)	0 (0.0)
Part-time	185 (23.0)	41 (6.9)	0 (0.0)
Full-time	498 (61.9)	484 (81.2)	8 (88.9)
Occupational retraining	0 (0.0)	1 (0.2)	0 (0.0)
Volunteer	2 (0.2)	0 (0.0)	0 (0.0)
Housewife/houseman	3 (0.4)	2 (0.3)	0 (0.0)
Parental leave	20 (2.5)	1 (0.2)	0 (0.0)
Still in school	3 (0.4)	1 (0.2)	0 (0.0)
Still in apprenticeship	17 (2.1)	6 (1.0)	0 (0.0)
Still in university	86 (10.7)	59 (9.9)	0 (0.0)
Employment ban	8 (1.0)	0 (0.0)	0 (0.0)
Occupational disability	3 (0.4)	0 (0.0)	0 (0.0)
Other	18 (2.2)	9 (1.5)	0 (0.0)
Missing data	11 (1.4)	7 (1.2)	1 (0.0)
Current employment			
No employment	17 (2.1)	5 (0.8)	0 (0.0)
Not regularly employed	2 (0.2)	3 (0.5)	0 (0.0)
Marginal employment	21 (2.6)	17 (2.9)	0 (0.0)
Part-time	135 (16.8)	44 (7.4)	1 (11.1)
Full-time	359 (44.6)	492 (82.6)	8 (88.9)
Occupational retraining	0 (0.0)	2 (0.3)	0 (0.0)
Volunteer	1 (0.1)	0 (0.0)	0 (0.0)
Housewife/houseman	9 (1.1)	1 (0.2)	0 (0.0)
Parental leave	103 (12.8)	1 (0.2)	0 (0.0)
Still in school	3 (0.4)	0 (0.0)	0 (0.0)
Still in apprenticeship	6 (0.7)	6 (1.0)	0 (0.0)
Still in university	62 (7.7)	47 (7.9)	0 (0.0)
Employment ban	239 (29.7)	0 (0.0)	0 (0.0)
Occupational disability	3 (0.4)	0 (0.0)	0 (0.0)
Other	24 (3.0)	8 (1.3)	0 (0.0)
Missing data	1 (0.1)	4 (0.7)	0 (0.0)

Multiple answers were possible.

to their child during the period from July 2017 to July 2018 with preterm delivery in $n = 13$ cases (2.8%) which is lower than in the overall German population (in 2013: 8.7%, Statistisches Bundesamt; born in hospitals in 2017: 8.6 %, IQTIG–Institut für Qualitätssicherung und Transparenz im Gesundheitswesen, 2018). $N = 465$ mothers gave birth to $n = 472$ children with a nearly balanced gender ratio (51.5%; $n = 243$ female) which is representative for the overall German population (48.7% female) and Saxony (48.9% female) in 2017 (Statistisches Bundesamt, 2018d). Most of the children were delivered vaginally (80.6%; $n = 375$). The ratio of cesarean sections (18.5%; $n = 86$) was as low as it is typical for the area of Dresden (in 2013: 19.5%; Statistisches Bundesamt, 2015), i.e., lower than in Saxony (24.0%) and in the overall German population (30.5%) in 2017 (Statistisches Bundesamt, 2018c). The majority of partners attended birth (97.3%; $n = 319$ male partners and 100%; $n = 3$ female partners).

Dropout Analyses

Dropout analyses (tables on request) considering sociodemographic characteristics of completers vs. non-completers (whose T2 data were due by the end of September 2018) were conducted separately for expectant mothers ($n = 465$ vs. $n = 96$) and male partners ($n = 328$ vs. $n = 90$), but not for female partners due to the small sample sizes ($n = 3$ vs. $n = 3$). Completers and non-completers at T2 did not differ in sociodemographic characteristics with some exceptions.

Regarding expectant mothers, completers were reporting German as their mother tongue (96.8 vs. 91.7%; Fisher's exact test, $p < 0.05$) and intended to take parental leave (99.3 vs. 95.5%; Fisher's exact test, $p < 0.01$) more often than non-completers. While there were no differences regarding current employment, completers differed from non-completers regarding employment 1 year ago. Formerly, completers were working in a full-time position more often than non-completers (65.4 vs. 53.7%; $\chi^2 = 4.674$, $df = 1$, $p < 0.05$), while the ratio of not being regularly employed (0.2 vs. 4.2%; Fisher's exact test, $p < 0.01$) and being a housewife (0.0 vs. 2.1%; Fisher's exact test, $p < 0.05$) was lower in completers than in non-completers.

Regarding male partners, completers lived permanently together with the expectant mother more often (96.3 vs. 88.8%; Fisher's exact test, $p < 0.05$), had a higher education (e.g., 61.6 vs. 51.1% having a subject-related or higher education entrance qualification (A-level); $U = 12113.0$; $z = -2.140$; $p < 0.05$), and a higher professional qualification (e.g., 57.0 vs. 40.0% having a university or doctoral degree; $U = 11458.0$; $z = -2.424$; $p < 0.05$) than non-completers.

DISCUSSION

Examining expectant mothers and their partners from pregnancy to at least 2 years postpartum, the DREAM study is one of the first prospective cohort studies investigating the changes of parental work participation and conditions, role distributions, and stress factors and its impact on perinatal outcomes and long-term mental and somatic health of the entire family while taking into account underlying biological processes regarding long-term activity of the HPA axis.

Altogether, major strengths of the DREAM study are (1) the prospective design stretching from one measurement point prepartum to at least three additional measurement points within the first two years postpartum (prolongation into middle childhood planned) which allows both longitudinal and cross-sectional analyses, (2) a unique multi-method approach combining quantitative data (using predominantly well-established scales) with long-term endocrinological data ($\text{DREAM}_{\text{HAIR}}$) and qualitative interview data ($\text{DREAM}_{\text{TALK}}$), (3) a large, representative community sample (interim sample of $N = 1{,}410$ participants by the end of September 2018,

TABLE 5 | Comparisons between sexes and first-time vs. parents already having children regarding full-time and part-time work status 1 year ago, i.e., prior to pregnancy (T1).

	Compared groups	Work status	*n (%)*	χ^2	*df*	*p*
Comparison between sexes	EM_0 vs. P_0	Full-time	EM_0: 446 (72.1%) P_0: 360 (80.4%)	9.702	1	0.00
		Part-time	EM_0: 102 (16.5%) P_0: 32 (7.1%)	20.626	1	0.00
	EM_{1+} vs. P_{1+}	Full-time	EM_{1+}: 48 (28.4%) P_{1+}: 111 (87.4%)	101.521	1	0.00
		Part-time	EM_{1+}: 81 (47.9%) P_{1+}: 9 (7.1%)	57.160	1	0.00
Comparison between first-time parents and parents already having children	EM_0 vs. EM_{1+}	Full-time	EM_0: 446 (72.1%) EM_{1+}: 48 (28.4%)	108.140	1	0.00
		Part-time	EM_0: 102 (16.5%) EM_{1+}: 81 (47.9%)	73.648	1	0.00
	P_0 vs. P_{1+}	Full-time	P_0: 360 (80.4%) P_{1+}: 111 (87.4%)	3.314	1	0.07
		Part-time	P_0: 32 (7.1%) P_{1+}: 9 (7.1%)	0.000	1	0.98

At T1 (during pregnancy), participants were asked whether they have been working full-time or part-time 1 year ago, i.e., prior to pregnancy. EM0: first-time expectant mothers; EM1+: expectant mothers already having children; P0: first-time fathers (male partners); P1+: fathers (male partners) already having children.

TABLE 6 | Birth-related characteristics as reported by the mothers 8 weeks after anticipated birth date (T2).

	Mothers ($n = 465$) *n (%)*
Number of delivered children	
One	459 (98.7)
Twins	5 (1.1)
Multiples	1 (0.2)
Preterm delivery	
Yes	13 (2.8)
No	445 (95.7)
Missing data	7 (1.5)
Mode of delivery	
Spontaneous vaginal birth	257 (55.3)
Vaginal birth induced by drugs	81 (17.4)
Vaginal operative birth (with forceps or vacuum extraction)	37 (7.9)
Planned cesarean section due to personal reasons	2 (0.4)
Planned cesarean section due to medical reasons	31 (6.7)
Unplanned cesarean section	53 (11.4)
Missing data	4 (0.9)
Sex of child ($n = 472$)	
Female	243 (51.5)
Male	221 (46.8)
Missing data	8 (1.7)

targeting $N = 4{,}000$ participants) of both mothers and male or female partners allowing the use of advanced statistical approaches such as multi-level modeling, (4) comparison to a clinical sample of mothers with postpartum mental disorders, their children, and (if available) their partners during the course from admission to discharge of a MBU tracking intervention effects and post-treatment, (5) a cooperation within Germany and abroad with matching instruments and assessment waves to facilitate internal and cross-cultural comparisons of findings.

Regarding the interim sample, first results of analyses conducted for this paper showed that our interim sample mainly consists of expectant mothers and their partners who expect their first child. This is because some of the recruitment ways like the clinics' information evenings or birth preparation courses are visited mainly by families expecting their first child. Nevertheless, it is exactly those parents who are of main interest, as the present study aims primarily at investigating the transition and change from being a couple to becoming a family. Further, there are slightly more expectant mothers than partners participating and the majority of participants has a rather high educational and professional level. This is in accordance with previous findings, i.e., people are more likely to participate in epidemiologic studies if they are female, well-educated, and/or have a higher socioeconomic status (e.g., Søgaard et al., 2004; Galea and Tracy, 2007; Gustavson et al., 2012). The self-selection bias might be even bigger in family studies. First, fathers might join or complete those studies more seldom than mothers. Second, particularly those fathers who do participate might be more involved in family matters or might have more progressive gender role values than nonparticipating fathers as discussed in Costigan and Cox (2001). Also, it is difficult to reach and recruit becoming fathers who are not in a relationship with the becoming mother or who are not accompanying their partners at the information evening or birth preparation courses. Moreover, it is conceivable that families interested in the compatibility of work and family may have contributed to the high ratio of expectant mothers and partners holding a full-time position. When speaking about the representativeness of the sample and generalizability of findings, the possibility of a healthy worker-effect has to be

considered, i.e., in research on working populations, healthy and little burdened individuals are often overrepresented due to their greater likelihood of participating in workforce (Li and Sung, 1999; Shah, 2009).

Further, we showed that a higher ratio of first-time mothers has been working full-time prior to pregnancy compared to mothers already having a child/children. No such difference was found in male partners. This is in accordance with previous findings that German women but not men reduce their amount of working time after giving birth to a child (Bundesministerium für Familie Senioren Frauen und Jugend, 2017a). More importantly, this is in line with our postulation of an emerging gender gap interfering with gender equality as it has consequences for long-term labor market inclusion/exclusion (Barker and Pawlak, 2011; Miani and Hoorens, 2014). Altogether, this sample seems to be appropriate for examining our research questions as a pronounced number of working participants is needed to profoundly investigate the changes of parental work participation, role distributions, and stress factors and its long-term implications for family health.

Regarding birth-related characteristics, first analyses showed that the ratio of mothers who delivered by cesarean section is as low as typical for the region (Statistisches Bundesamt, 2015) while the rate of preterm deliveries of the interim DREAM sample is lower compared to the overall German population (Statistisches Bundesamt, 2015; IQTIG–Institut für Qualitätssicherung und Transparenz im Gesundheitswesen, 2018). A possible reason may be that participating mothers who had a preterm delivery were more likely to drop out because child care is more extensive in the early postpartum, i.e., an already sensitive period.

Dropout analyses found only a few differences between completers and non-completers at T2 indicating that (1) non-completing male partners had a lower educational level as well as professional qualification and lived more seldom permanently together compared to completing male partners and (2) non-completing mothers were speaking German as their mother tongue, working full-time, and planning to take parental leave less often than completing mothers. However, the DREAM study is characterized by a rather low dropout rate which is supported by our retention strategy using reminders and incentives (Booker et al., 2011). In future, we will still increase personal contact via telephone to participants who do not send back the follow-up questionnaires in time to reduce the attrition even more. This will be an additional strength in the further course of the study as we prospectively would like to add measurement points reaching into middle childhood. Hence, it will be possible to examine long-term effects on family health as well as on a potential gender gap regarding labor market inclusion/exclusion (Schober and Zoch, 2015).

To sum up, the DREAM study will contribute to a better understanding of the complex relationships between parental work participation, role distribution, and stress factors and its implications for perinatal outcome and long-term mental and somatic health of mothers, their partners in parenting, and children. With former studies neglecting confounding and moderating factors, this innovative study with its sub-studies allows a comprehensive picture of the family as a whole. To our knowledge, it is the first study combining work-related health implications with the assessment of long-term activity of the HPA axis as measured by HCC in each individual and parent-child dyad. In particular, examining the cumulated steroid hormones of both parents and their child in HCC data, the current findings will be valuable for understanding the transgenerational transmissions of long-term alterations of the HPA axis and the interplay as a couple. The comparison to a clinical sample of mothers, their children, and (if available) their partners who will be assessed during the course of a state-of-the-art treatment in a MBU will help to better understand the potential underlying mechanisms of these processes and intervention benefits. The clinical sub-study has further the potential to add evidence regarding the role of parent-child interaction for child health, particularly its temperament and regulation disorders. Moreover, it is unique to this research area to investigate both mother-child and father-child dyads. Finally, results will not only be of scientific interest but also of socio-political relevance as they will generate important findings warranted by this inter-disciplinary research field and thus may contribute moving these issues higher on the political agenda.

ETHICS STATEMENT

This study has been carried out in accordance with the recommendations of the Ethics Committee of the Faculty of Medicine of the Technische Universität Dresden with written consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. This protocol was approved by the DREAM steering group and by the Ethics Committee of the Faculty of Medicine of the Technische Universität Dresden (No: EK 278062015).

AUTHOR CONTRIBUTIONS

SG-N has acquired the funding and been responsible for conception and design of the basic DREAM study with its sub-studies as well as the coordination and supervision of the (ongoing) data collection. JJ-H, SS-S, SG-N, and AF have been responsible for the conception and design of the sub-study $DREAM_{HAIR}$. MK, VK, CA, CS, and AF supported the conduction of the study, especially through data collection. PW provided access to potential participants. KW provided resources for the acquisition of data in the DREAM study. CK advised the sub-study $DREAM_{HAIR}$ and provided support for hair cortisol analyses. MK and VK prepared the data for statistical analysis. VK performed the statistical analysis except from Monte Carlo simulations. TvS performed Monte Carlo simulations. VK wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

The DREAM study is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation;

GA 2287/4-1) and by the Deutsche Gesellschaft für Psychosomatische Frauenheilkunde und Geburtshilfe (DGPFG e.V., German Society of Psychosomatic Obstetrics and Gynecology).

ACKNOWLEDGMENTS

We want to thank all (expectant) mothers and their partners for supporting our project. Furthermore, we want to thank all cooperating clinics and midwives for providing access to the potential participants as well as all students performing the recruitment. We wish to extend many thanks to all institutions supporting our lottery. We are further very grateful to Dr. Janice Hegewald who as a native English-speaking researcher back-translated the mentioned questionnaires.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2019.01273/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 12 June 2019
doi: 10.3389/fpsyg.2019.01319

Estradiol Fluctuation, Sensitivity to Stress, and Depressive Symptoms in the Menopause Transition: A Pilot Study

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The menopause transition is associated with an increased risk of depressed mood. Preliminary evidence suggests that increased sensitivity to psychosocial stress, triggered by exaggerated perimenopausal estradiol fluctuation, may play a role. However, accurately quantifying estradiol fluctuation while minimizing participant burden has posed a methodological challenge in the field. The current pilot project aimed to test the feasibility of capturing perimenopausal estradiol fluctuation via 12 weekly measurements of estrone-3-glucuronide (E1G), a urinary metabolite of estradiol, using participant-collected urine samples in 15 euthymic perimenopausal women ages 45–55 years. Furthermore, it aimed to correlate E1G fluctuation (standard deviation across the 12 E1G measurements) with weekly mood and cardiovascular, salivary cortisol, and subjective emotional responses to the Trier Social Stress Test (TSST) at weeks 4, 8, and 12. Protocol acceptability and adherence was high; furthermore, E1G fluctuation was positively associated with anhedonic depressive symptoms and weekly negative affect. E1G fluctuation was also associated with increased heart rate throughout the TSST as well as higher levels of rejection, anger, and sadness. E1G fluctuation was not significantly associated with TSST blood pressure or cortisol levels. This study suggests a feasible method of assessing estradiol fluctuation in the menopause transition and provides support for the hypothesis that perimenopausal estradiol fluctuation increases sensitivity to psychosocial stress and vulnerability to depressed mood.

Keywords: menopause transition, perimenopausal depression, estradiol, trier social stress test, estrone-3-glucuronide

OPEN ACCESS

Edited by:
Beate Ditzen, Heidelberg University Hospital, Germany

Reviewed by:
Reuma Gadassi-Polack, Yale University, United States
Harriet De Wit, The University of Chicago, United States

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Specialty section:
This article was submitted to Psychology for Clinical Settings, a section of the journal Frontiers in Psychology

Received: *08 November 2018*
Accepted: *20 May 2019*
Published: *12 June 2019*

Citation:
Gordon JL, Peltier A, Grummisch JA and Sykes Tottenham L (2019) Estradiol Fluctuation, Sensitivity to Stress, and Depressive Symptoms in the Menopause Transition: A Pilot Study. Front. Psychol. 10:1319. doi: 10.3389/fpsyg.2019.01319

INTRODUCTION

The menopause transition (a.k.a. *perimenopause*) represents the reproductive stage transitioning from regular menstrual cycles through the loss of ovulatory function and to the complete cessation of menses. The latter marks the onset of menopause. Between ages 42 and 55, nearly all women experience the menopause transition, which, on average, extends 5–6 years preceding the last menstrual period (Treloar, 1981; Oldenhave et al., 1993; Avis and McKinlay, 1995). A recent review article identified 12 cross-sectional studies comparing rates of elevated depressive symptoms in pre- and peri-menopausal women and concluded that 45–68% of perimenopausal women, versus only 28–31% of premenopausal women, report clinically significant elevations in depressive symptoms (Maki et al., 2019).

Rates of *diagnosed* perimenopausal Major Depressive Disorder based on DSM-IV criteria (American Psychiatric Association, 2000) range between 12 and 23% (Cohen et al., 2006; Bromberger et al., 2011). Lost work productivity and medical costs associated with perimenopausal depression are estimated at $10,000 USD/woman/year (daCosta DiBonaventura et al., 2012).

Despite the substantial burden that perimenopausal depression places on millions of women, little is known about the biological mechanisms underlying its etiology. However, it has been hypothesized that the hormonal environment characterizing the menopause transition may play a role (Schmidt and Rubinow, 2009; Freeman, 2010). As a woman progresses through the menopause transition, menstruation becomes increasingly unpredictable and ovulation becomes increasingly rare – at the endocrine level, levels of progesterone, which rise following ovulation, become progressively more stable. In contrast, research comparing daily hormone levels in reproductive-aged and perimenopausal women have confirmed that the menopause transition is characterized by more extreme levels of estradiol (E2) than would be seen in a typical menstrual cycle; for example, luteal phase E2 levels have been shown to be higher in the menopause transition, sometimes reaching levels that are even double those generally seen in the late follicular phase (Hale et al., 2007; Hale and Burger, 2009). Furthermore, E2 levels in the early follicular phase have been shown to reach lower levels than typically observed in reproductive-aged women (Shideler et al., 1989). Several factors are believed to contribute to these more extreme E2 levels, including extreme fluctuation in follicle stimulating hormone, which controls the development of E2-producing follicles (i.e., eggs) in the ovaries, and greater variability in the number of follicles available for stimulation (O'Connor et al., 2003; Santoro and Randolph, 2011; Hale et al., 2014). It is theorized that increased E2 fluctuation – that is, repeated exposure to the rapid shift between the above-mentioned lower E2 "lows" and higher E2 "highs" than typically seen among reproductive-aged women – may play a key role in the etiology of perimenopausal depression (Gordon et al., 2015). Such a hypothesis would be consistent with the findings of a recent trial in which 172 euthymic perimenopausal women were randomized to receive either an E2 patch (0.1 mg), which would serve to reduce E2 fluctuation, or a placebo patch, for 12 months. Overall, women assigned to placebo were more likely to develop clinically significant depressive symptoms [score $\geq$16 on the Center for Epidemiologic Studies – Depression Scale (CES-D)] when compared to women assigned to E2 (odds ratio = 2.5).

To our knowledge, six studies have directly examined the relationship between natural fluctuations in E2 levels and mood in perimenopausal women. However, three of the six studies measured E2 levels less than once per year, likely contributing to their null findings (Avis et al., 2001; Woods et al., 2008; Bromberger et al., 2011). The fourth study, the Penn Ovarian Aging Study (Freeman et al., 2006) of 231 mid-life women, measured E2 levels twice per year, 1 month apart, over 8 years, and calculated E2 variability as the standard deviation associated with the two E2 measurements. In that study, years characterized by greater variability in E2 were associated with an increased risk of developing clinical elevations in depressive symptoms and major depressive disorder. The two most recent studies (Gordon et al., 2016a,b) measured E2 with greater frequency, therefore providing a more direct test of whether it is E2 fluctuation rather than another epiphenomenon of menstrual irregularity that triggers perimenopausal depressive symptoms. The first study (Gordon et al., 2016b), examined the relationship between depressive symptoms and perimenopausal E2 fluctuation using four blood samples over the course of 14 months and found that the standard deviation across the four E2 measurements was positively associated with depressive symptoms at the end of the study among 20 women who had recently experienced a stressful life event. In the second study (Gordon et al., 2016a), salivary E2 levels and mood were concurrently assessed once weekly for four weeks among 30 perimenopausal women. The results of this pilot study revealed that greater change in E2 from one week to the next – particularly a greater increase in E2 – was associated with a subsequent increase in overall depressive symptoms, sadness, hopelessness, guilt, anger, anxiety, and feelings of social rejection. These results, in combination with the previously described studies, may suggest that greater mood sensitivity to acute changes in E2 is involved in the development of perimenopausal depression.

While the mechanisms by which E2 fluctuation may increase the risk of perimenopausal depression is unknown, increased sensitivity to psychosocial stress has been proposed as a possibility (Gordon et al., 2015). In one of the above-mentioned studies finding a significant relationship between E2 flux over 14 months and the emergence of perimenopausal depressive symptoms among women reporting at least one very stressful life event at baseline (Gordon et al., 2016b), E2 flux also predicted increased negative emotional responses to a standardized psychosocial stressor battery – the Trier Social Stress Test (TSST) – particularly exaggerated feelings of anger and rejection. These findings may suggest that increased E2 fluctuation in the menopause transition increases women's sensitivity to stress; when this increased sensitivity is combined with stressful life events, depression ensues (**Figure 1**).

Indeed, the concept that increased sensitivity to stress might contribute to a vulnerability to developing perimenopausal depression is consistent with the broader literature suggesting that increased stress sensitivity may precede and contribute to risk for major depressive disorder unrelated to reproductive events (Pariante and Lightman, 2008). For example, hypercortisolism has been shown to precede the onset of first-episode major depressive disorder in high-risk adolescents (Goodyer et al., 2000). Furthermore, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis following successful depression treatment has been shown to predict relapse (Appelhof et al., 2006). The observation that euthymic relatives of individuals with a history of depression exhibit hypercortisolism (Mannie et al., 2007) further contributes to the view that stress axis dysregulation may be a risk factor for depression rather than simply a consequence or epiphenomenon of depression. However, while compelling, this hypothesized

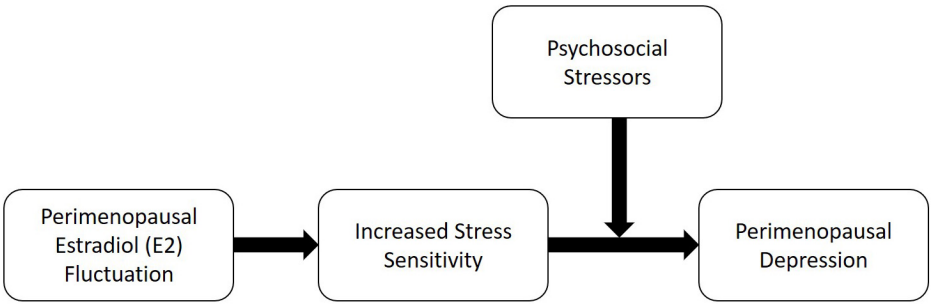

FIGURE 1 | Theoretical model of perimenopausal depression development.

model of perimenopausal depression development requires additional testing.

Rationale for the Current Study

Further research is needed to clarify the role that perimenopausal hormonal fluctuation and increased stress sensitivity play in triggering perimenopausal depressive symptoms as well as the ways in which these variables interact with the psychosocial environment to which a woman is exposed to predict depression risk. However, measuring reproductive hormone levels with sufficient frequency to adequately capture E2 fluctuation while minimizing participant burden and dropout presents an important methodological challenge, likely explaining why studies to date have assessed E2 levels and mood a maximum of four times. Plasma or serum E2 levels require repeated in-clinic venipuncture, making this a painful, and burdensome option for participants. While measuring E2 in participant-collected saliva samples is considerably more convenient, there is evidence suggesting that salivary E2 correlates only modestly with E2 levels in blood (Shirtcliff et al., 2000), particularly when used to detect levels that are as low as those sometimes seen in perimenopausal women (Tivis et al., 2005). Furthermore, E2 stability in saliva is relatively low and prone to deterioration with repeated freeze-thaw cycles (Lewis, 2006), making it important to maintain freezing temperatures when transporting saliva from participants' homes. The current pilot study therefore aimed to assess the feasibility of capturing perimenopausal E2 fluctuation using a urinary metabolite of E2, estrone-3-glucuronide (E1G) – in 12 weekly participant-collected urine samples. Pregnanediol glucuronide (Pdg), a urinary metabolite of progesterone, was also measured to be included as a covariate in all analyses. These metabolites have been shown to correlate very highly (rs = 0.93–0.97) with serum levels of E2 and progesterone measured 1 day prior to urine collection (O'Connor et al., 2003). In other words, first-morning urine levels of E1G and PdG reflect an integrated measure of the overall hormone levels from the previous day. Furthermore, because urine can be non-invasively collected by participants at home, it represents an attractive alternative to blood.

A second goal of the current pilot study was to examine whether E2 fluctuation, measured using the above-mentioned methodology, would be associated with responses to a psychological laboratory stressor and/or a failure to habituate to such a stressor, administered multiple times. Thus, in addition to measuring weekly mood for 12 weeks, the current study administered the TSST – a highly structured and well-validated psychosocial stress protocol (Kirschbaum et al., 1993; Allen et al., 2014) – at weeks 4, 8, and 12. It was hypothesized that women exhibiting greater E1G fluctuation over the course of the 12 weeks would exhibit greater physiological and negative emotional responses to the TSST as well as demonstrate a failure to habituate to repeated administrations of the TSST. In addition, it was hypothesized that within-person analyses would reveal a significant relationship between greater weekly absolute change in E2 and both negative mood and greater responses to the TSST.

MATERIALS AND METHODS

Participants

Fifteen medically healthy women were recruited who were aged 45–55 years and perimenopausal according to the Stages of Reproductive Aging Workshop (STRAW +10) criteria (early perimenopause, defined as menstrual cycle length 7+ days longer than usual; late perimenopause, defined as ≥2 skipped cycles and an interval of amenorrhea ≥60 days but within 1 year of last menstrual period) (Harlow et al., 2012). Exclusion criteria included the following: depressive symptoms in the clinically significant range, as defined as a CES-D score of 16 or above (Radloff, 1977; Thomas et al., 2001), currently using medications affecting mood or ovarian hormone levels (e.g., antidepressants and oral contraceptives), pregnant, or nursing. To ensure safety during stress testing, participants could not have a diagnosis of cardiovascular disease or hypertension or a resting blood pressure >140/90 at enrollment.

The study was advertised through flyers posted throughout Regina as well as through advertisements on social media. Participants were compensated $220 for completing the study. This research project was reviewed and approved by the University of Regina Research Ethics Board.

Study Overview

Participants first underwent an enrollment visit during which their study eligibility was determined and informed written

consent was obtained. At this time, participants completed questionnaires assessing detailed medical and medication history, demographic characteristics, and depressive symptoms. If determined eligible for the study, urine collection supplies were given to the participant to take home and detailed instructions on urine collection were given. Once weekly for 12 weeks, participants were emailed online mood surveys, and were reminded to collect a first-morning sample of urine the following day. On weeks 4, 8, and 12, on the day of mood survey completion (the day prior to urine collection), participants attended an in-person stress testing session in the laboratory during which emotion ratings, cortisol, heart rate, and blood pressure were assessed. On stress testing days, it was ensured that mood surveys were completed prior to stress testing. **Figure 2** depicts the overall study design.

Weekly Mood and Hormone Measurement

Depressive symptoms were assessed once weekly using the Center for Epidemiologic Studies- Depression Scale (CES-D), a 20-item self-report form that asks about the frequency of depressive symptoms during the previous week on a 4-point scale of 0 (rarely) to 3 (most or all of the time) (Radloff, 1977). A score of 16 or above is commonly used as a cut-off for identifying potential clinical depression (Boyd et al., 1982) and is predictive of major depression (Thomas et al., 2001). Three subscales of the CES-D – somatic symptoms (items 1, 2, 5, 7, 11, and 20), negative affect (items 3, 6, 14, and 18) and anhedonia (items 4, 8, 12, and 16) (Carleton et al., 2013) – were also examined in the current study. The CES-D has been frequently used in perimenopausal samples (Avis and McKinlay, 1995; Daly et al., 2003; Freeman et al., 2006; Woods et al., 2008; Bromberger et al., 2011).

Positive and negative affect was also evaluated using the PANAS-X (Positive and Negative Affect Schedule – Expanded Form) (Watson and Clark, 1999). Participants rated the extent to which they endorse 60 emotions *right now* on a 5-point Likert scale, one being "very slightly or not at all" and five being "extremely." The PANAS-X is one of the most widely used instruments in mood research; its validity and reliability as a measure of positive and negative affect have been well-established through rigorous statistical and time-spanning tests (Bagozzi, 1993).

Because first-morning voided urine levels of E1G and PdG reflect an integrated measure of the overall hormone levels from the previous day (O'Connor et al., 2003), urine collection occurred on the morning following the assessment of mood. On urine collection days, participants used the provided supplies to collect a sample of their first-morning voided urine in a plastic cup and used a syringe to fill one 2-ml polypropylene tube, which they placed in their home freezer in a tube storage box. This protocol was repeated on the same day every week for 12 weeks.

Once all urine samples had been collected by the participants, they were retrieved by a research assistant and taken to the laboratory at the University of Regina. Because urinary E1G and PdG concentrations are not affected by the repeated freezing and thawing of specimens (O'Connor et al., 2003), an icepack was used to simply keep the samples cool as they were taken to the laboratory within 2 h of pick up. Once received, samples were frozen at −40°C until they were assayed, which occurred within 45 days of receiving them.

Hormonal Assays

Estrone-3-glucuronide, a urinary metabolite of estradiol, was assayed using an enzyme immunoassay (Arbor Assays, Ann Arbor, MI, United States), with sensitivity at <22.5 pg/ml. The specificity is high, showing ≤0.1% cross-reactivity with similarly, structured compounds. Its cross-reactivity with estradiol is somewhat higher, however, at 5%. The intraassay coefficient of variation was 5.1% and the interassay coefficient of variation was 14.8%. PdG was also assayed using an enzyme immunoassay (Arbor Assays, Ann Arbor, MI, United States), with sensitivity at <0.180 ng/ml. The specificity is high, showing ≤0.2% cross-reactivity with similarly, structured compounds. Its cross-reactivity with 20-α-hydroxyprogesterone and 20-β-hydroxyprogesterone is somewhat higher, however, at 45 and 3.2%, respectively. However, the concentration of these compounds were expected to be excessively small in the samples. The intraassay coefficient of variation was 9.1% and the interassay coefficient of variation was also 9.1%. To account for differences in urine concentration, E1G, and PdG levels were adjusted for specific gravity using the formula recommended by O'Connor et al. (2003).

The Trier Social Stress Test (TSST)

At study weeks 4, 8 and 12, participants came to the laboratory to undergo the TSST, which has been shown to induce a reliable stress response (Kirschbaum et al., 1993). All laboratory sessions

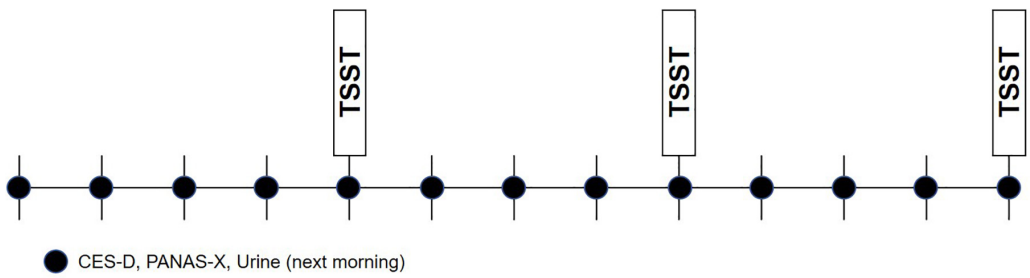

FIGURE 2 | Study design.

began between 2:00 pm and 5:00 pm to minimize the effect of diurnal changes in cortisol (Allen et al., 2014) and all three sessions were booked at the same time for each participant. Each session began with a 30-min rest period during which participants read magazines in a quiet room. The TSST involved four components:

(1) Pre-task instructions (1 min): participants were introduced to the committee who later listened to their speech and were given instructions for the mental arithmetic task;
(2) Speech preparation period (3 min): participants prepared their speech while the selection committee stood in the room;
(3) Speech (5 min): immediately following the preparation period, the selection committee asked the participant to deliver her speech. If the participant ended her speech before 5 min, the selection committee questioned her in a systematic fashion to ensure she spoke the entire 5 min; and
(4) Serial subtraction task (5 min): a 1-digit number was subtracted from a 4-digit number as fast and as accurately as possible for 5 min. For each mistake, the participant was instructed by a member of the selection committee to restart from the beginning.

Participants were video-recorded throughout their performance. To minimize habituation to this task, the exact speech topic, and instructions differed for each of the three laboratory stress sessions (job interview, promotion, and award nomination), as did the numbers involved in the mental arithmetic task, the location of the stress task, and the researcher administering the test.

Mood Measurements During the TSST

As recommended by Hellhammer and Schubert (2012), participants were asked to complete brief emotion rating scales every 10 min during the baseline period, following the speech task instructions, following the speech task, following the serial subtraction task, and every 10 min of the recovery period of the laboratory stress session. Specific emotions assessed included stress, sadness, anger, and feelings of rejection. The scale is anchored with 0 being "not at all" and 10 being "extremely."

Physiological Measurements During the TSST

Blood pressure and heart rate were obtained at: minutes 20, 22, 24, 26, and 28 of the baseline period; minutes 0 and 2 of the speech preparation period, the speech, and the arithmetic task; and at minutes 2, 4, 8, 10, 13, 16, 20, 25, 30, 40, 45, 56, and 60 of the recovery period. These measures were then averaged to obtain a mean baseline, preparation, speech task, arithmetic task, and recovery value for each measure.

Saliva samples were collected for cortisol measurement at the end of the 30-min baseline rest period as well as minutes 0, 15, and 60 of the recovery period, aimed at capturing the peak in cortisol, which occurs 20–30 min after stressor onset (Allen et al., 2014). Salivary cortisol was determined using a Cortisol Enzyme Immunoassay Kit (Salimetrics) processed at the University of Regina SPIT Laboratory. Intra- and inter-assay coefficients of variation were low at 5.0 and 2.9%, respectively. The minimum cortisol detection level with this assay is 0.007 ug/dl.

Data Management and Analysis

PROC MIXED in SAS 9.4 was used to carry out two sets of analyses – the first examining the within-person effect of weekly E1G fluctuation and the second examining the between-person effect of E1G fluctuation across all 12 weeks. In both cases, models were fitted using a restricted maximum likelihood (REML) estimation method, which is well-suited for small samples (Peugh, 2010). A first-order autoregressive covariance structure for within-person error was applied and the Kenward-Rogers correction was used to calculate the appropriate degrees of freedom.

For analyses testing the within-person effect of E1G fluctuation on weekly mood and stress test outcomes, the following fixed factors were included in the regression model: (1) absolute-value change in E1G since the previous week; (2) the direction of the change in E1G since the previous week; and (3) the interaction between these two variables. In addition, E1G and PdG levels on the day of outcome measurement (measured in urine the day after outcome measurement) were included as covariates.

For analyses testing the between-person effect of E1G fluctuation, the standard deviation in E1G across the 12 weekly measurements was examined in relation to weekly mood as well as responses to all three administrations of the TSST. Again, E1G and PdG levels on the day of outcome measurement (measured in urine the day after outcome measurement) were included as covariates.

For between-subject analyses examining stress testing outcomes, an additional model tested the interaction between the standard deviation in E1G and stress testing week (4, 8, or 12) to evaluate whether E1G fluctuation predicted habituation to repeated administrations of the TSST.

All estimates reported throughout the manuscript reflect the quantity of change in the dependent variable associated with 1 standard deviation's worth of change in the independent variable.

RESULTS

Participant Characteristics

The reproductive and hormonal characteristics of the 15 study participants are presented in **Table 1**. All but one woman was Caucasian and all were high school graduates, with 6/15 having a university degree. The mean gross household income was $90,000–112,999. The ages ranged from 45 to 54 years. All participants scored below 16 on the CES-D, with baseline scores ranging from 2 to 13.

Estrone-3-glucuronide fluctuation across the 12-week study (calculated as the standard deviation in E1G levels) was significantly correlated with a participant's maximum E1G level [$r(15) = 0.99$, $p < 0.0001$], mean E1G level [$r(15) = 0.97$, $p < 0.0001$], maximum PdG level reached [$r(15) = 0.68$, $p = 0.006$], and mean PdG [$r(15) = 0.56$, $p = 0.032$]. However,

TABLE 1 | Reproductive and hormonal participant characteristics.

Age (years)	Perimenopausal stage	# Months since LMP	Minimum E1G (pg/ml)	Maximum E1G (pg/ml)	E1G SD (pg/ml)	Minimum PdG (pg/ml)	Maximum PdG (pg/ml)
52	Late	3	1,115	4,577	1,129	27	180
52	Late	1	3,171	9,501	2,012	143	803
53	Late	0	3,511	11,640	2,324	233	848
46	Late	5	3,946	11,940	2,399	249	663
46	Late	5	341	9,138	2,866	26	842
54	Late	10	5,487	16,840	3,378	175	874
46	Late	4	3,655	35,920	8,954	111	444
47	Early	0	2,891	42,830	13,334	124	2,315
50	Late	4	2,802	54,690	15,551	94	1,940
46	Early	1	14,970	61,350	15,815	231	5,750
44	Late	5	4,167	93,910	28,082	240	4,723
47	Late	2	7,747	122,200	35,568	634	3,757
45	Early	0	10,970	133,100	41,181	389	8,320
50	Late	0	5,188	139,300	43,456	94	987
47	Late	1	5,826	191,800	55,972	216	5,074

Note: LMP: last menstrual period.

E1G fluctuation was not correlated with either minimum E1G [r(15) = 0.43, p = 0.104] or minimum PdG level reached [r(15) = 0.44, p = 0.101].

Protocol Compliance

Overall protocol adherence was high: participants completed 100% of the required weekly urine samples and 84% (10 out of 12) of the weekly mood surveys. In three instances, the day on which the weekly urine sample and mood survey occurred were changed to accommodate participants' schedules (e.g., being out of town for a part of the week). All but one participant completed all three stress testing sessions; this participant was withdrawn from the stress testing portion of the study after her blood pressure reached concerningly high levels in the first session. Her blood pressure values from the first stress testing session were not included in any analyses.

Overall Efficacy of the Stress Protocol

A significant effect of stress testing phase was found for systolic blood pressure [F(4,136) = 30.9, p < 0.0001], diastolic blood pressure [F(4,127) = 32.5, p < 0.0001], heart rate [F(4,77) = 7.9, p < 0.001], and subjective stress levels [F(6,223) = 28.1, p < 0.0001] such that levels during the preparation, speech, and arithmetic phases were significantly higher than baseline levels (ps < 0.05; **Figure 3**). Similarly, a significant effect of stress testing phase was found for cortisol [F(3,106) = 8.5, p < 0.0001] such that both the second and third samples (but not the fourth) were significantly greater than the baseline sample (ps < 0.05). Non-significant effects of TSST administration number (ps > 0.05) and non-significant phase-by-administration number interaction (ps > 0.05) indicate that participants demonstrated a similar cardiovascular, cortisol, and subjective stress response across all three TSST administrations.

Within-Person Effects of E1G Fluctuation

Table 2 depicts the results of analyses investigating the within-person effect of E1G fluctuation on responses to the TSST and weekly mood, which included on-the-day E1G and PdG levels as covariates. The results suggest that change in E1G levels from one week to the next, regardless of the direction of the change, was associated with higher heart rate and anger in response to the TSST. In addition, a drop in E1G from one week to the next was associated with more negative affect and tended to be associated with a higher total CES-D score, a higher CES-D anhedonia subscale score, as well as higher diastolic blood pressure during the TSST. The effect of hormone levels were non-significant for the most part, with the exception of a positive effect of E1G and negative effect of PdG on anger.

Between-Person Effects of E1G Fluctuation on Responses to the TSST

Physiological Responses

Adjusting for E1G and PdG levels on the day of the TSST, E1G fluctuation across the twelve-week study was associated with greater heart rate [β(SE) = 6.6(2.9), p = 0.020] (**Figure 4**) and diastolic blood pressure [β(SE) = 1.8(1.0), p = 0.056] (**Figure 5**) but not systolic blood pressure [β(SE) = -0.4(1.2), p = 0.809] or cortisol [β(SE) = 0.0(0.0), p = 0.296] throughout stress testing. E1G fluctuation did not interact with stress testing phase (ps > 0.05) or week (ps > 0.05) to predict any cardiovascular variables or cortisol.

Emotional Responses

Adjusting for on-the-day E1G and PdG levels, greater E1G fluctuation over the entire 12 weeks predicted greater and overall feelings of rejection [β(SE) = 0.3(0.0), p < 0.001], anger [β(SE) = 0.3(0.0), p < 0.0001], but not stress [β(SE) = 0.3(0.2), p = 0.094] in response to the TSST. While there was also a significant relationship between

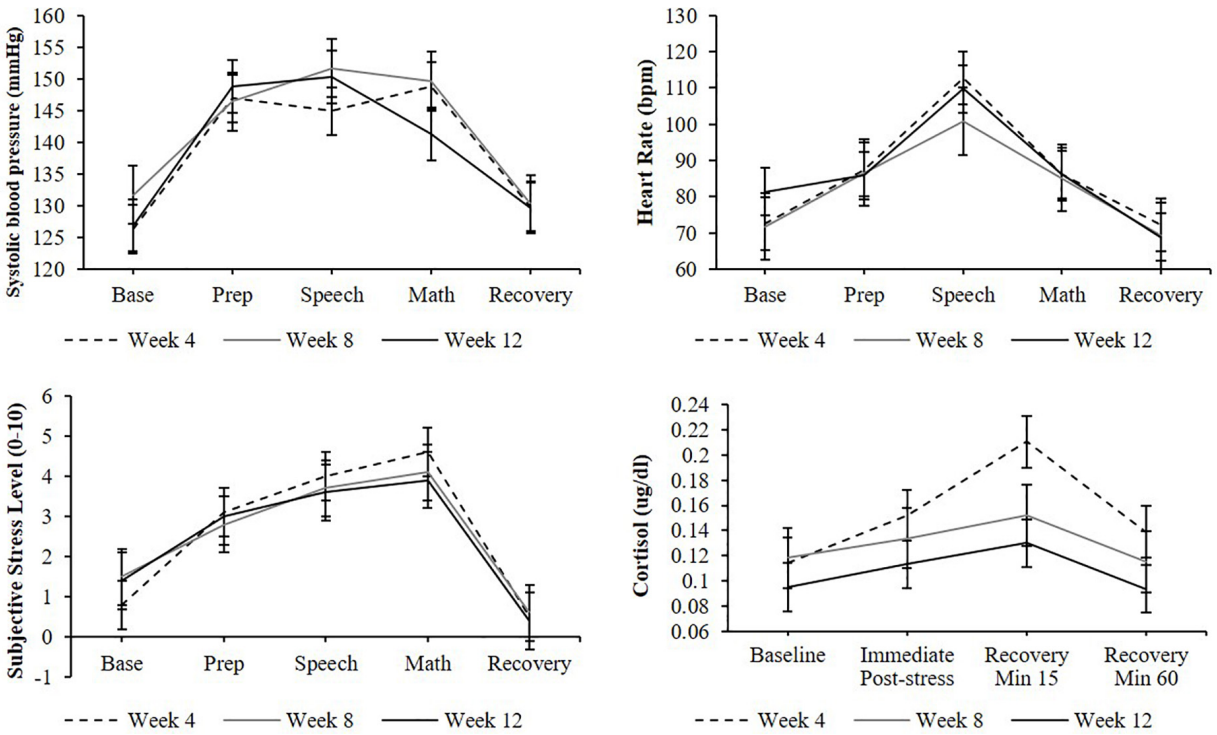

FIGURE 3 | Cardiovascular, cortisol, subjective stress responses to stress testing, and administered at weeks 4, 8, and 12. Standard error bars shown.

TABLE 2 | Within-person effects of weekly absolute value change in E1G, absolute value E1G change by change direction, E1G level, and PdG level on weekly mood and responses to the TSST.

Variable	β(SEM) |ΔE1G| [a]	β(SEM) |ΔE1G| X direction[b]	β(SEM) E1G level	β(SEM) PdG level
Physiological responses to the TSST				
Heart rate	3.0 (5.5)*	−3.0 (6.3)	−1.4 (4.6)	2.0 (2.7)
Systolic blood pressure	−2.4 (2.4)	−2.4 (2.7)	2.0 (2.0)	0.3 (1.2)
Diastolic blood pressure	1.9 (2.1)	−3.5 (2.1)#	2.1 (1.6)	0.9 (0.9)
Cortisol AUC	1.1 (2.3)	−0.5 (0.5)	0.2 (1.9)	1.2 (1.1)
Emotional responses to the TSST				
Rejection	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	−0.1 (0.0)
Anger	0.3 (0.1)*	0.2 (0.1)	0.4 (0.1)**	−0.3 (0.0)**
Stress	0.1 (0.4)	−0.2 (0.4)	−0.6 (0.3)#	0.0 (0.2)
Sadness	0.0 (0.1)	−0.1 (0.1)	0.0 (0.1)	0.1 (0.0)
Weekly mood				
Negative affect	1.0 (0.7)	−1.5 (0.7)*	1.1 (0.7)	0.1 (0.7)
Positive affect	0.5 (1.0)	−0.3 (1.1)	1.0 (1.1)	0.2 (0.6)
CES-D total	0.5 (0.9)	−1.9 (1.9)#	0.9 (0.9)	0.1 (0.5)
CES-D negative affect	0.1 (0.2)	−0.3 (0.3)	0.2 (0.2)	0.1 (0.1)
CES-D anhedonia	0.2 (0.3)	−0.7 (0.4)#	0.6 (0.4)	−0.3 (0.2)
CES-D somatic	0.1 (0.3)	−0.1 (0.4)	−0.1 (0.4)	0.1 (0.2)

*[a]Indicates the magnitude of the change in the dependent variable that is associated with 1 SD change in E1G (in either direction). [b]Indicates the difference in the effect of 1 SD of change in E1G when the direction of the change from the previous week is up vs. down. A negative value indicates that the effect of a decline in E1G is stronger than an increase in E1G. #p < 0.10; *p < 0.05; **p < 0.01.*

E1G fluctuation and overall sadness [β(SE) = 0.3(0.0), $p < 0.0001$], a significant interaction between E1G fluctuation and stress testing phase ($p < 0.0001$) suggested that the effect of E1G fluctuation was only significant for the post-speech [β(SE) = 0.5(0.1), $p < 0.001$], and post-arithmetic [β(SE) = 0.7(0.1), $p < 0.0001$] assessments. E1G fluctuation did not otherwise interact with testing phase ($ps > 0.05$) and did not significantly interact with

administration number ($ps > 0.05$) to predict any emotional responses to the TSST.

Between-Person Effects of E1G Fluctuation on Weekly Mood

PANAS-X

Adjusting for E1G and PdG levels, greater E1G fluctuation over the entire 12 weeks, determined using the standard deviation in E1G levels, predicted greater weekly negative affect [β(SE) = 1.9(0.8), $p = 0.042$] but not positive affect [β(SE) = 0.7(1.8), $p = 0.691$] (**Figure 6**).

CES-D

Greater E1G fluctuation predicted higher scores on the anhedonia subscale of the CES-D [β(SE) = 0.8(0.3), $p = 0.016$] and a weak trend was seen between greater E1G fluctuation and a higher total CES-D score [β(SE) = 1.1(0.7), $p = 0.122$]. However, no effect of E1G fluctuation was seen on the somatic ($p = 0.349$) or the negative affect ($p = 0.548$) CES-D subscales (**Figure 7**).

DISCUSSION

The current study aimed to test the feasibility of capturing perimenopausal reproductive hormone flux using weekly urine samples for the measurement of estradiol and progesterone metabolites; furthermore, it examined the relationship between E1G fluctuation and mood (assessed using weekly questionnaires) and stress sensitivity (assessed using physiological and emotional responses to multiple administrations of the TSST). The feasibility of measuring reproductive hormone fluctuation using reproductive hormone metabolites was supported, as was the study protocol: participants were highly adherent to the weekly urine samples and mood surveys, and stress testing was successful in triggering a stress response, even upon the third administration. Results suggested that E1G fluctuation across the 12-week study was associated with more weekly negative affect and anhedonic depressive symptoms, as well as higher heart rate, diastolic blood pressure, and feelings of rejection, anger and sadness during the TSST. At a within-subjects level, greater change in E1G from one week to the next was associated with greater negative affect, as well as higher heart rate and anger during stress testing.

Reflecting on some of the methodological details that may have contributed to our study's success, we suspect that participant reminders by email, phone, or text (depending on the participant's preference) on the day prior to mood measurement and prior to urine collection were critical. Allowing participants to choose the day of the week that was most convenient for them and allowing flexibility for cases in which participants were going to be out of town was also helpful. Providing disposable cups and disposable syringes, allowing the participant to transfer urine from a large cup to the 2 ml polypropylene tube also seemed to contribute to participants' willingness to comply with the urine collection protocol. Related to the repeated administration of the TSST: the fact that all three stress testing sessions were administered not only using different speech and arithmetic task instructions but also by a different person and in a different location each time may have helped to minimize habituation to the stressor despite repeated administrations within a short timespan. One final practical detail we wish to mention for any researcher considering incorporating the measurement of urinary metabolites in their research relates to the method used to adjust metabolite levels according to the concentration of the urine. One commonly used method for doing so involves measuring and adjusting for urinary levels of creatinine, a by-product of muscle activity that is excreted in urine (Taussey, 1954). Our decision to use specific gravity with a refractometer, which provides a proxy of urine concentration by measuring the absorption of light through the sample, was based on two considerations: (1) adjusting for specific gravity has been found to be as effective as creatinine correction, and even more effective in cases of very dilute or highly concentrated urine (Miller et al., 2004); (2) in the long run, specific gravity would be more cost-effective as it requires a one-time purchase of a refractometer

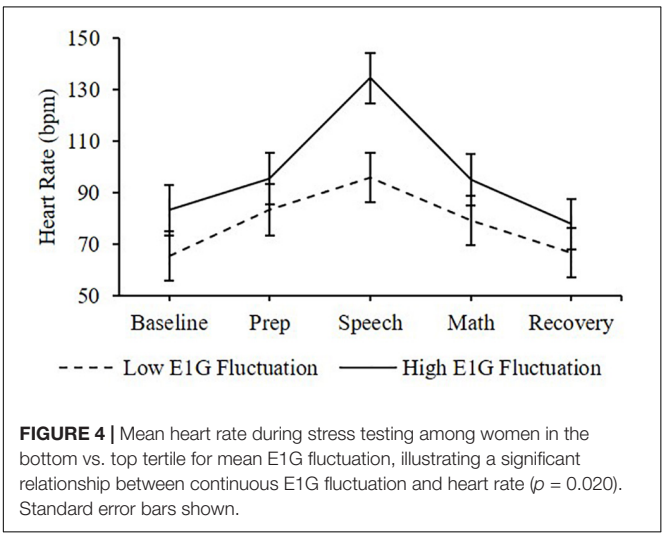

FIGURE 4 | Mean heart rate during stress testing among women in the bottom vs. top tertile for mean E1G fluctuation, illustrating a significant relationship between continuous E1G fluctuation and heart rate ($p = 0.020$). Standard error bars shown.

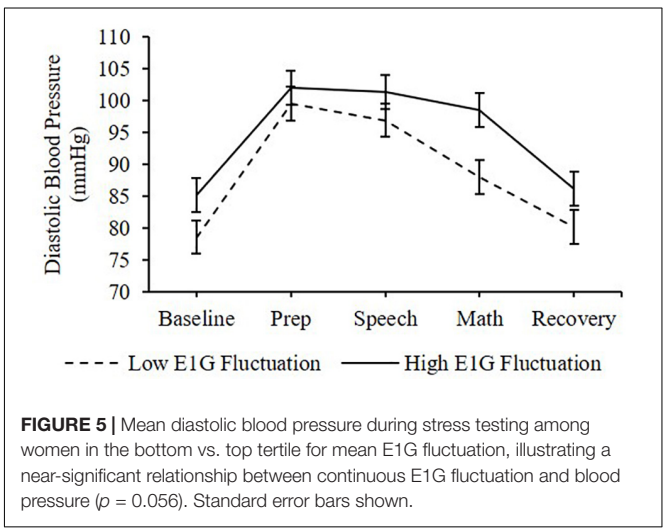

FIGURE 5 | Mean diastolic blood pressure during stress testing among women in the bottom vs. top tertile for mean E1G fluctuation, illustrating a near-significant relationship between continuous E1G fluctuation and blood pressure ($p = 0.056$). Standard error bars shown.

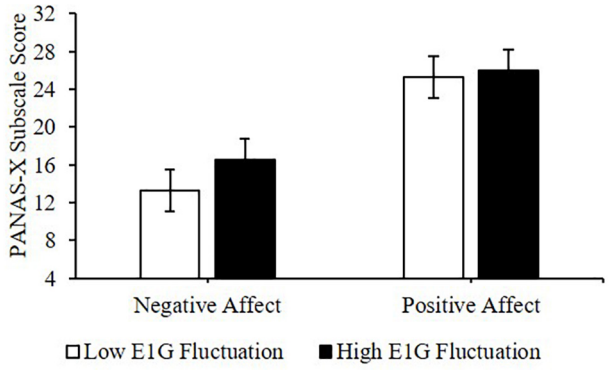

FIGURE 6 | Mean PANAS-X subscale scores among women in the bottom vs. top tertile for mean E1G fluctuation, illustrating a significant relationship between continuous E1G fluctuation, and weekly negative affect ($p = 0.042$) but not sadness ($p = 0.691$).

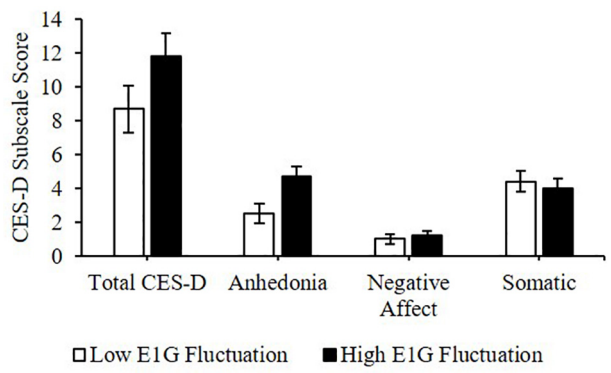

FIGURE 7 | Mean CES-D subscale scores among women in the bottom vs. top tertile for mean E1G fluctuation, illustrating a significant relationship between continuous E1G fluctuation, and the anhedonia subscale of the CES-D ($p = 0.016$) as well as a weak trend between greater E1G fluctuation and a higher total CES-D score ($p = 0.122$). No effect of E1G fluctuation was seen on the somatic ($p = 0.349$) or the negative affect CES-D subscale ($p = 0.548$). Standard error bars shown.

whereas creatinine correction requires the repeated purchase of creatinine assay kits; and (3) specific gravity is much less time-intensive, requiring fewer human resources.

Despite the small sample size and limited statistical power of this pilot study, we detected a significant relationship between E1G fluctuation and negative mood, indicated by the negative affect subscale of the PANAS-X and the anhedonia subscale of the CES-D. Importantly, the effect of E1G fluctuation on mood was independent of hormone *levels*. This is consistent with previous studies linking estradiol fluctuation with perimenopausal mood (Freeman et al., 2006; Gordon et al., 2016a,b) and consistent with the observation that depression risk decreases in the postmenopausal period (Freeman et al., 2004; Cohen et al., 2006; Bromberger et al., 2007, 2011; Woods et al., 2008), when estradiol levels are low but stable. Finally, it is consistent with the findings of a recent clinical trial comparing the efficacy of transdermal estradiol vs. placebo in preventing depressive symptoms in perimenopausal and early postmenopausal women (Gordon et al., 2018): in this trial, the benefits of estradiol were most apparent in the early perimenopausal women when compared to the late perimenopausal and early postmenopausal women. Since early perimenopausal women show the highest mean E2 levels but also the highest E2 fluctuation, these findings suggest that the beneficial effects of transdermal E2 were mediated by its E2 *stabilization* effects rather than by *increasing* E2. It is noteworthy that although all participants in the current study were in the menopause transition, the range of E1G fluctuation was considerable, suggesting large individual variability in the amount of ovulatory activity occurring over the 12-week study. Our findings therefore suggest that although the overall risk for depressive mood is increased in the menopause transition, periods of relatively less ovulatory activity do occur and are accompanied by less negative mood.

Between-subject E1G fluctuation was also associated with higher heart rate and diastolic blood pressure, as well as feelings of rejection, sadness, and anger in response to the TSST, consistent with the sole previous study examining the effect of estradiol fluctuation on responses to the TSST (Gordon et al., 2016b). However, the effect of E1G fluctuation appeared to be similar across all three stress test administrations, contrary to our hypothesis that women with greater E1G fluctuation would show less habituation than women experiencing less fluctuation. Furthermore, the fact that the effect of E1G fluctuation did not interact with stress testing phase to predict most outcomes (apart from sadness) raises the possibility that greater E1G fluctuation may be associated with greater general resting-state arousal, perhaps resulting from heightened negative mood, rather than an increased sensitivity to stress that contributes to an increased vulnerability to depression. Further research is needed to clarify whether this increased stress sensitivity is a mechanism mediating the relationship between estradiol fluctuation and perimenopausal depressive symptoms or whether it is a consequence of hormonally triggered depressive symptoms. Experimental research directly manipulating estradiol levels and examining its effects on stress reactivity would help clarify the direction of this relationship.

The mechanisms by which estradiol fluctuation may increase sensitivity to stress and risk for depressed mood remain to be clarified. Candidate mechanisms underlying the negative mood effects of acute drops in estradiol include withdrawal from estradiol's anti-inflammatory (Vegeto et al., 2008), neuroprotective (Bredemann and McMahon, 2014), and serotonergic (Rubinow et al., 1998) effects. However, there is also evidence suggesting that a subset of the population – particularly women with current (Gordon et al., 2016a) or past (Jacobs et al., 2015) depression – may be especially sensitive to acute *increases* in estradiol. While the mechanisms underlying this effect are largely unknown, one postmortem study observing that women with major depressive disorder at the time of their deaths had lower estradiol receptor α expression in the frontal

cortex and hippocampus suggests that the altered expression and distribution of estradiol receptors in limbic and frontal regions may be involved (Perlman et al., 2005). It has also been suggested that the effect of estradiol fluctuation on mood may be mediated by fluctuations in neurosteroids (steroids that are produced *de novo* in the brain) that are modulated by estradiol. For example, allopregnanolone is a progesterone-derived neurosteroid that exerts both anxiolytic (Bitran et al., 1995) and antidepressant (Rodrìguez-Landa et al., 2007) effects via its GABAergic effects and is positively modulated by estradiol (Bernardi et al., 2003; Pluchino et al., 2005, 2009). Research conducted in rodents suggests that large fluctuations in allopregnanolone can reverse its psychological effects such that it becomes anxiogenic rather than anxiolytic (Shen et al., 2007). The fact that estradiol fluctuation was not related to self-reported stress levels or cortisol in the current study does not fully support the involvement of this mechanism; however, it should not be ruled out given our limited statistical power.

In considering the mechanisms linking estradiol fluctuation with perimenopausal mood, it should be emphasized that it is likely that the processes involved in mediating estradiol's effects on mood may vary from woman to woman, thus making individual women differentially sensitive to estradiol change in one direction or the other – this would be consistent with research observing a high degree of individual variability both in the magnitude and direction in sensitivity to reproductive hormone change across the menstrual cycle (Eisenlohr-Moul et al., 2016). The possibility that individual women may be differentially sensitive to changes in E2 – in both direction and magnitude – may help explain why many of the within-person effects of E1G fluctuation were found to be non-significant. The methods used in this pilot study, in a larger sample of women, may prove useful in examining individual differences in sensitivity to E2 change.

The current study findings should be interpreted in light of some limitations. First, the small sample size raises questions about the generalizability of the findings and limits our ability to examine the moderation of estradiol's effect on mood, such as life stress or depression history. Second, although the use of once-weekly samples may be sufficient in capturing between-person effects of hormonal fluctuation on overall mood, it is an imperfect method for capturing the acute estradiol changes that can impact mood. Daily or every-other-day measurements may be better suited for such purposes; however, the risk of overburdening participants and therefore increasing participant non-adherence and dropout must be weighed against the advantages of measuring hormone levels with greater frequency. The BIMORA study is one study that successfully used daily urine samples – over five six-month collection intervals – to examine reproductive hormone trajectories across the menopause transition (Ferrell et al., 2005). However, it is noteworthy that only 35% of eligible women agreed to participate in BIMORA and study retention was only 63%, which may be particularly problematic in a study on perimenopausal depression since we would likely expect women to be less adherent in the context of elevated depressive symptoms. The protocol used in the current study may therefore be an acceptable middle ground that balances participant burden and the accuracy with which E2 fluctuation is measured. Third, excluding women with elevated depressive symptoms at baseline limits the range of the outcome variables assessed and may limit the generalizability of the current study's findings to non-euthymic women. The inclusion of exclusively euthymic women may help explain why effects of E1G fluctuation were seen on the negative affect subscale of the PANAS but not of the CES-D, as the latter assesses more severe depressive mood. Finally, including premenopausal and postmenopausal women in the current study would have allowed us to compare mood and stress reactivity and their relation to hormonal fluctuation across the reproductive lifespan.

In conclusion, the current pilot study suggests a protocol that may be useful for investigating the role of estradiol fluctuation in the development of perimenopausal depressive symptoms. Furthermore, our findings suggest that periods of relative ovarian inactivity and estradiol stability in the menopause transition are associated with less self-reported negative mood and decreased sensitivity to psychosocial stress in a laboratory setting. Further research is needed to confirm whether perimenopausal depressive symptoms are more prone to waxing and waning than typical depression, depending on the hormonal environment to which a woman is being exposed at any given time.

ETHICS STATEMENT

The protocol was approved by the University of Regina Research Ethics Board. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

JeG, AP, and LST contributed to conception and design of the study. JeG and JuG performed the statistical analysis. JeG wrote the first draft of the manuscript. AP and JuG wrote sections of the manuscript. LST conducted the hormone assays. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

Funding for Open Access provided by the University of Regina President's Publication Fund. Funding for hormone assays and participant compensation provided by the University of Regina Dean of Arts Research Award, the University of Regina President's Research Seed Grant and the University of Regina Center on Aging and Health. JeG was also supported by a Tier II Canadian Institutes of Health Research (CIHR) Canada Research Chair.

ACKNOWLEDGMENTS

We thank Candice Giesinger, Fakhra Shahid, and Rashell Wozniak for their help with data collection.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 13 March 2019
doi: 10.3389/fpsyg.2019.00546

Psychobiological Factors of Sexual Functioning in Aging Women – Findings From the Women 40+ Healthy Aging Study

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Background: A variety of biological and psychosocial factors are associated with women's sexual health in midlife and older age. Evidence suggests a decline in sexual functioning in the context of aging and the menopausal transition, including changes in sexual desire, arousal, lubrication, orgasm, pain, and/or contentment. However, not all women in midlife and older age experience such a decline, and it remains unclear how the endocrine environment and psychosocial aspects contribute to the maintenance of healthy sexual functioning. Therefore, the aim of this study was to examine psychobiological predictors of sexual functioning in healthy middle-aged and elderly females.

Methods: A total of 93 healthy, sexually active women aged 40–73 years completed a battery of validated psychosocial questionnaires, including measures of sexual functioning (Female Sexual Function Index) and of protective psychological traits and interpersonal variables. The steroid hormones estrogen, testosterone, progesterone and dehydroepiandrosterone sulfate were determined in saliva samples, while follicle-stimulating hormone, luteinizing hormone and sex hormone-binding globulin were determined in dried blood spots. The findings were statistically adjusted for multiple testing.

Results: Age and postmenopausal status were negatively associated with overall sexual functioning, arousal, and lubrication. Regression analyses revealed that relationship satisfaction, emotional support, self-esteem, optimism, and life satisfaction each significantly predicted overall sexual functioning or specific aspects of sexual functioning, including arousal, contentment, orgasm, and pain (all $p < 0.029$). For desire and lubrication, no associations were found with the tested psychosocial factors. In terms of steroid hormones, testosterone was positively linked to orgasm ($p = 0.012$). In this sample, 79.6% reported to have healthy sexual functioning according to the questionnaires' cutoff. Younger age (OR = 0.911, 95% CI 0.854–0.970, $p = 0.004$) and a higher level of emotional support (OR = 1.376, 95% CI 1.033–1.833, $p = 0.029$) were associated with the presence of healthy sexual functioning.

OPEN ACCESS

Edited by:
Sophie Schweizer, Universität Heidelberg, Germany

Reviewed by:
Hatta Sidi, National University of Malaysia, Malaysia
Jennifer Lee Gordon, University of Regina, Canada

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Specialty section:
This article was submitted to Clinical and Health Psychology, a section of the journal Frontiers in Psychology

Received: *15 October 2018*
Accepted: *25 February 2019*
Published: *13 March 2019*

Citation:
Mernone L, Fiacco S and Ehlert U (2019) Psychobiological Factors of Sexual Functioning in Aging Women – Findings From the Women 40+ Healthy Aging Study. Front. Psychol. 10:546. doi: 10.3389/fpsyg.2019.00546

Discussion: Although aging and menopause negatively affected aspects of sexual functioning, the accompanying endocrine correlates were not predictive for sexual functioning in this healthy sample of middle-aged and older females. Instead, our findings suggest that sexual functioning is highly dependent on psychosocial aspects related to well-being. Accordingly, personality traits such as optimism, and interpersonal aspects such as emotional support and relationship satisfaction were identified as important predictors of sexual functioning.

Keywords: sexual functioning, female sexuality, women's health, midlife and older age, biopsychosocial factors

INTRODUCTION

As populations are aging rapidly all over the world, there has been a growing interest in healthy aging and associated factors. One important aspect that is often overlooked or treated as taboo in aging populations is sexual health (Lusti-Narasimhana and Beard, 2013), even though sexuality is considered to be a fundamental part of being human and a central component of general health (Graugaard, 2017). According to the definition of the World Health Organization, sexual health refers to "a state of physical, emotional, mental, and social well-being in relation to sexuality, and not merely the absence of disease, dysfunction, or infirmity" (World Health Organization, 2006, p. 5). There is considerable knowledge concerning the positive association of sexual health with general well-being and health-related quality of life (Davison et al., 2009; Lee et al., 2016; Greenberg et al., 2017).

Historically, it was assumed that sexuality and its associated physical response does not differ between men and women (Masters and Johnson, 1966). Accordingly, physical sexual response is characterized by the linear progression from excitement, to plateau, orgasm, and finally resolution. However, this classical model was frequently criticized due to its sole focus on the physical (genital) response and was therefore extended with the aspect of sexual desire preceding the excitement phase (Kaplan, 1979). Despite this adaptation, it has been debated whether this linear model of sexual response adequately reflects women's sexual experience. Basson (2001) proposed an alternative circular model, which specifically refers to the female's sexual response. This model emphasizes the importance of emotional intimacy (as a result of a satisfying sexual experience) for future willingness to engage in sexual activity. According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), sexual functioning encompasses a complex interplay of biological, psychological, and sociocultural factors and can be significantly disturbed when a person's ability to respond sexually or experience sexual pleasure is impaired (American Psychiatric Association, 2013).

Women's sexual functioning can vary, or permanently change, across the life course, especially in the context of reproductive events and aging (Clayton and Harsh, 2016). There is a growing body of research investigating sexual functioning in middle-aged women experiencing the menopausal transition. Most of these studies have found a decline in sexual functioning in association with the menopausal transition (Dennerstein et al., 2003; Avis et al., 2017), with hormonal changes and associated vaginal dryness assumed to be the key factors in this decline. Moreover, studies have also looked at the aging process independently of menopausal status. Sexual functioning is thought to decline with increasing age. This may be due to an overall decline in general health as women age, physical changes or medical conditions (Clayton and Harsh, 2016). The age-associated decline in sexual functioning has been underpinned by prevalence studies of sexual dysfunction in middle and older age. For example, Nicolosi et al. (2004) assessed prevalence rates of sexual dysfunction in approximately 14,000 women aged 40–80 years from 29 countries. More than 65% of all women reported to have engaged in sexual activity in the past year. Among these, 38% reported having sexual intercourse more than once a week. The most prevalent sexual dysfunction in middle-aged and elderly women was a lack of sexual interest (21%), followed by inability to reach orgasm (16%) and lubrication difficulties (16%), no pleasure during intercourse (15%), and pain during intercourse (10%). A clear age pattern was detected, with increased prevalence rates with higher age (Nicolosi et al., 2004). According to an elaborated overview of the worldwide prevalence of sexual dysfunction provided by McCabe et al. (2016), the prevalence rates for low levels of female sexual desire vary between 17 and 55%. Negative age effects were observed after the age of 60, when the prevalence rates mostly range between 40 and 50%. Arousal difficulties have often been operationalized as lubrication insufficiencies, with varying prevalence rates of 21 to 28%. The prevalence of orgasmic dysfunction varies between 16 and 37%. Pain during sexual activity seems to be less prevalent in the general population, ranging from 1 to 27% across various studies (McCabe et al., 2016).

In sum, sexual dysfunction seems to increase in midlife and older age, including changes in sexual desire, arousal, lubrication, orgasm, pain, and/or contentment. However, there is a high numerical variability across different studies and countries, and there are some methodological issues which limit the generalizability of the findings (Hayes and Dennerstein, 2005). Furthermore, there is no consensus regarding the aspects of sexual functioning that change with advancing age. It is important to acknowledge, however, that not all women in midlife and older age experience a decline in sexual functioning. In a study by Trompeter et al. (2012), most of the surveyed sexually active women aged between 40 and 99 years reported frequent arousal, lubrication, and orgasm, even if sexual desire was low. Additionally, sexual satisfaction was higher in older women and was not associated with sexual

activity. Lonnèe-Hoffmann et al. (2014) found similar results, demonstrating no significant changes in sexual functioning in women who maintained their sexual activity from early to late postmenopause. These and other studies reveal that sexual functioning does not necessarily worsen over the course of aging and that research to date has mainly focused on sexual dysfunction and negative aspects of sexuality among elderly women (Lusti-Narasimhana and Beard, 2013). So far, studies focusing on sexual health and positive aspects of sexual functioning are sparse. A focus on sexual function rather than dysfunction might be a more favorable approach in the investigation of females' sexual health. Insights into factors associated with the maintenance of healthy sexual functioning could potentially be of considerable benefit in terms of positive health research.

As reflected in the WHO definition of sexual health, which takes various dimensions into consideration and emphasizes the role of well-being (World Health Organization, 2006), sexual functioning should be defined positively and within a biopsychosocial framework (Graugaard, 2017). Such a perspective on sexual health in midlife and older age has been established in the past few years (Conklin, 2017). A central issue in the investigation of female sexual functioning is the importance of other factors besides menopausal status and age (e.g., Dennerstein et al., 2003). Psychosocial factors, particularly psychological and interpersonal variables, seem to play a crucial role in middle-aged and elderly women's sexual functioning (DeLamater and Karraker, 2009; Thomas and Thurston, 2016). As presented in **Figure 1**, biological determinants include, among other factors, age, menopausal status, and hormonal changes related to menopause and aging (Fiacco et al., 2018). In particular, sex steroids can exert an impact on sexual functioning due to the fluctuations which occur with aging and in the context of the menopausal transition (Clayton and Harsh, 2016). Estradiol (E2) is the best-examined sex steroid with regard to sexual functioning in middle and older age. E2 levels show a sharp decline during the menopausal transition, causing vaginal atrophy, dryness and irritation, which may indirectly lead to decreased sexual desire, arousal, and response (Wierman et al., 2010). Moreover, androgens also seem to be associated with female sexual functioning. Testosterone (T) is the sex steroid which is assumed to primarily influence sexual desire and motivation (Bachmann and Leiblum, 2004; Wåhlin-Jacobsen et al., 2015). However, the role of estrogens and androgens in relation to female sexual functioning is highly controversial, since findings are inconsistent and many studies did not report any associations (Meston and Frohlich, 2000; Wierman et al., 2010). For dehydroepiandrosterone sulfate (DHEA-S), which is the most abundant sex steroid in females and an androgen precursor, mostly positive associations with sexual functioning have been reported (e.g., Davis et al., 2005). Little attention has been paid to progesterone (P), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone-binding globulin (SHBG) in relation to female sexual functioning (Dennerstein et al., 2005; Randolph et al., 2015; Worsley et al., 2016). To conclude, more research is required in order to further examine the contribution of sex steroids to women's sexual functioning in midlife and older age, and non-hormonal factors also need to be taken into account.

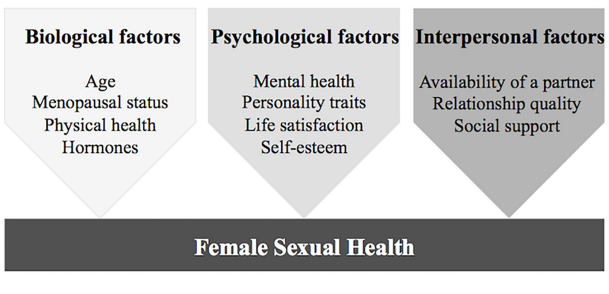

FIGURE 1 | Biopsychosocial model of female sexual health in midlife and older age: biological, psychological, and interpersonal factors potentially contributing to healthy sexual functioning.

As illustrated in **Figure 1**, psychological factors determining sexual functioning may include good mental health (absence of psychiatric conditions), specific psychological factors related to well-being such as self-esteem or life satisfaction, and protective personality traits. Psychiatric disorders constitute a risk factor for female sexual dysfunction (Basson and Gilks, 2018), with depression and anxiety in particular showing negative associations with sexual functioning (Althof et al., 2005). Therefore, the absence of mental disorders such as depression or anxiety may be protective for female sexual health. Another psychological factor related to subjective well-being that may have a positive impact on sexual functioning is general life satisfaction. To date, only a small number of studies have pointed out the relevance of life satisfaction for sexual functioning in women (Woloski-Wruble et al., 2010; Stephenson and Meston, 2015). Other studies have found a high level of self-esteem to be a positive psychological factor in female sexuality (Goodson et al., 2006; Rehbein-Narvaez et al., 2006). Additionally, personality traits seem to be significant for sexual health: A recent meta-analysis linking the Big Five personality traits (McCrae and John, 1992) to sexual health showed that a high level of extraversion was associated with fewer symptoms of sexual dysfunction and a higher frequency of sexual activity (Allen and Walter, 2018). Moreover, a higher degree of extraversion was also found to be related to greater sexual satisfaction (Allen and Desille, 2017) and to a higher level of orgasmic frequency and overall sexual functioning (Harris et al., 2008; Crisp et al., 2015).

Finally, besides biological and psychological determinants of sexual functioning in midlife and older age, interpersonal factors are crucial in the investigation of female sexual health (**Figure 1**). First, the availability of a partner is an important factor for the engagement in sexual activity and therefore sexual functioning (DeLamater and Karraker, 2009). If they have a partner, the majority of middle-aged and older women remain sexually active (Thomas et al., 2015). The general satisfaction with one's partner or quality of communication in the relationship are crucial for females in order to experience satisfying sexuality

(Byers, 2005; Thomas and Thurston, 2016). Social support from one's partner may be another interpersonal factor which affects sexuality due to its health-promoting effects (Uchino, 2006), but this has not yet been investigated in the context of women's aging. Finally, the partner's physical or mental health status may have an impact on females' sexual functioning (DeLamater and Karraker, 2009).

To summarize, it can be stated that first, female sexuality in midlife and older age has often been studied in terms of dysfunction and risk factors. Little attention has been given to factors that may predict female sexual health in midlife and older age. Hence, the present study focused on aspects of sexual functioning instead of dysfunction, and on associated protective factors. Furthermore, we investigated exclusively healthy women, since studies with a solely healthy female sample are scarce. Second, a biopsychosocial approach that simultaneously considers biological, psychological, and interpersonal factors should be applied when investigating female sexual functioning. The questions of what the most important determinants of sexual health in midlife and older age are, and how psychosocial aspects and the endocrine environment in these stages of life contribute to the maintenance of healthy sexual functioning, have not been investigated extensively. Therefore, the aim of this study was to examine psychobiological predictors of sexual functioning in a healthy sample of middle-aged and elderly females. For this purpose, we examined the associations of protective psychological and interpersonal factors, as well as endocrine factors (sex hormones), with female sexual functioning and its various components.

MATERIALS AND METHODS

This study was part of the Women 40+ Healthy Aging Study, a large research project that was conducted at the Department of Clinical Psychology and Psychotherapy of the University of Zurich. The goal of the research project was to investigate healthy middle-aged and older women using a biopsychosocial framework.

Study Participants and Procedure

In total, 130 self-reporting healthy women aged 40–73 years participated in the study. The sample was recruited among the general population using online advertisements and flyers. Participants' self-reported health status was used as inclusion criteria. The participants had to report either a good, very good, or excellent health condition, and had to state that they were currently free of any acute or chronic somatic disease or mental disorder. Furthermore, none of the participants had received psychotherapy or psychopharmacological treatment in the previous 6 months. Additional exclusion criteria were applied regarding the assessment of hormones: pregnancy in the last 6 months; precocious menopause or menopausal status due to surgical removal of either both ovaries or the uterus; current use of oral contraceptives or use of hormone therapy in the last 6 months; any disease influencing the endocrine system; current diabetes mellitus, polycystic ovary syndrome, hirsutism, or endometriosis. Our study population included women with pre-, peri-, and postmenopausal status. According to the frequently used classification system *Stages of Reproductive Aging Workshop +10* (Harlow et al., 2012), we considered women with regular menstrual cycle as premenopausal; women with variable cycle length or an interval of amenorrhea of >60 days as perimenopausal; and women without menstrual bleeding in the last 12 months or more as postmenopausal. As illustrated in the participant flow chart (**Figure 2**), 37 women had to be excluded from the total sample for the data analyses. Most of these women reported not having had any sexual activity in the last month, which is a prerequisite for the appropriate use of the instrument measuring female sexual functioning (Rosen et al., 2000). Unfortunately, some women showed inconsistencies when reporting whether they engaged in sexual activity and therefore had to be excluded. Therefore, the total and final sample for the present analyses amounted to $N = 93$ healthy and sexually active women.

The study procedure consisted of two parts: In a first step, participants were invited to the laboratory for a physiological assessment, including the standardized collection of biological material for the analyses of endocrine parameters. Second, they completed validated psychosocial questionnaires online. In order to control for the menstrual cycle phase in premenopausal women, the physiological and psychosocial assessments were applied in the follicular phase. Written informed consent was obtained from all participants prior to data collection. The Cantonal Ethics Committee (KEK) of the canton of Zurich and the Cantonal Data Protection Commission of the canton of Zurich approved the study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Psychosocial Measures

Psychosocial measures included protective psychological traits and interpersonal factors. All psychosocial measures were assessed online via self-reports using validated questionnaires. The participants were explicitly informed about the procedure and expected duration (approximately 30 min) of the online assessment prior to filling in the questionnaires. It was required to complete all questionnaires consecutively.

Sexual Functioning

Sexual functioning was examined using the German version of the Female Sexual Function Index (FSFI; Rosen et al., 2000) validated by Berner et al. (2004). The FSFI is a multidimensional self-report instrument and is considered to be the gold standard for the assessment of female sexual functioning. It contains 19 items covering six key domains of female sexual functioning: desire, arousal, lubrication, orgasm, satisfaction, and pain. Each domain comprises two to four items assessing the frequency, the level (degree) of, and/or satisfaction with the corresponding sexual functioning domain. Response options varied depending on the type of item. For items measuring frequency, the response options ranged from 0 (*no sexual activity*) to 5 (*almost always or always*). Items measuring the level of the specific sexual functioning domain or the satisfaction with it were either rated as

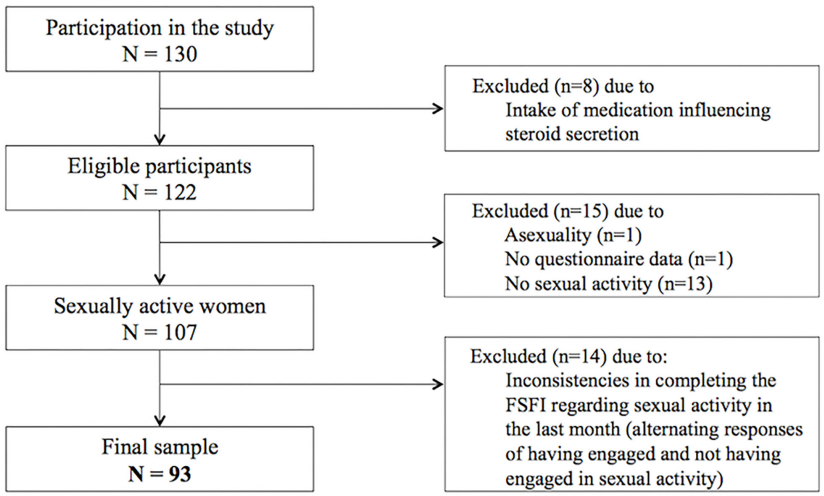

FIGURE 2 | Participant flow chart.

0 (*no sexual activity*) or rated from 1 (*very low or none at all/very dissatisfied*) to 5 (*very high/very satisfied*). To obtain individual domain scores for each sexual functioning domain, the scores of the corresponding items were added up and multiplied by the domain factor. Additionally, a full-scale score (total FSFI score) was calculated by summing up the six domain scores. Full-scale scores range from 2 to 36, with higher scores indicating better overall sexual functioning. According to Wiegel et al. (2005), women with and without sexual dysfunction can be discriminated by a total FSFI cutoff value of 26.55. Regarding the psychometric properties of the German version of the FSFI, Berner et al. (2004) reported satisfactory results in their validation study, with internal consistencies for the FSFI domains and total score ranging from $\alpha = 0.75$ (satisfaction) to $\alpha = 0.95$ (pain). In the present study, we found an excellent internal consistency for the total FSFI score (Cronbach's alpha = 0.92) and a range from $\alpha = 0.74$ (satisfaction) to $\alpha = 0.91$ (pain) for the FSFI domains. The estimated duration to complete the FSFI is approximately 10 min (Berner et al., 2004).

Protective Psychological Markers

Self-esteem

The Multidimensional Self-Esteem Scale (MSES; Schütz and Sellin, 2006) was used to assess overall self-esteem. This self-report scale comprises a total of 32 items rated on a 7-point Likert scale, which examine six different aspects of self-esteem: emotional self-esteem, social skills, social confidence, achievement-related self-esteem, physical attractiveness, and sportiness. Furthermore, a global self-esteem score can be computed which includes all dimensions, and for which satisfactory reliability and validity (Cronbach's alpha = 0.93) have been reported (Schütz and Sellin, 2006). This global score was used in the present study; internal consistency in the present study was acceptable (Cronbach's alpha = 0.77). It takes approximately 10–15 min to complete the MSES (Schütz and Sellin, 2006).

Optimism

Dispositional optimism was measured with the German version of the Life Orientation Test-Revised (LOT-R) developed by Glaesmer et al. (2008). The LOT-R encompasses 10 items (three items each for optimism and pessimism, four neutral items) that are rated on a 5-point Likert scale. The item scores are added up to build an optimism and a pessimism scale. Only the optimism scale was used in this study. Glaesmer et al. (2008) reported an internal consistency of $\alpha = 0.69$ for the optimism scale. Despite this rather questionable value, the authors argue that factor analysis clearly supports a two-factor structure and that the scale is applicable for research purposes. This is also supported by the short duration required to complete the LOT-R (approximately 5 min).

Extraversion

The short version of the Big Five Inventory (BFI-K; Rammstedt and John, 2005) was used to evaluate extraversion. The BFI-K contains 21 items rated on a 5-point Likert scale and depicts the Big Five personality traits: extraversion, neuroticism, conscientiousness, agreeableness, and openness. To build the trait subscales, the corresponding four to five item scores per trait are added together. In this study, only the extraversion subscale was used. Rammstedt and John (2005) reported satisfactory psychometric properties of the BFI-K, with an internal consistency ranging between $\alpha = 0.81$ and 0.86 for the extraversion scale in different samples. The Cronbach's alpha of extraversion in this study lay at 0.79, implying an acceptable internal consistency. The BFI-K is a particularly economic instrument, since the average duration to complete all items is under 2 min (Rammstedt and John, 2005).

Life satisfaction

Participants' life satisfaction was measured with the most commonly used instrument in this area, the Satisfaction with Life Scale (SWLS; Glaesmer et al., 2011). The SWLS comprises five items rated on a 7-point Likert scale and is completed within few

minutes. In a German validation study, Glaesmer et al. (2011) found very good internal consistency for the SWLS (Cronbach's alpha = 0.92). Cronbach's alpha in the present study lay at 0.87.

Interpersonal Factors

Relationship satisfaction

The Relationship Assessment Scale (RAS; Sander and Böcker, 1993) was used to evaluate participants' satisfaction with their intimate relationship. The RAS is a brief measure, only comprising seven items rated on a 5-point Likert scale. Validation studies found satisfactory results regarding reliability and validity of the instrument (Sander and Böcker, 1993). In the present study, the internal consistency of the RAS was excellent, with a Cronbach's alpha of 0.91.

Emotional support

Emotional support was assessed using the Berlin Social Support Scales (BSSS; Schwarzer and Schulz, 2000). The BSSS measures the level of emotional support, instrumental support, search for social support, and need for social support using 17 items rated on a 4-point Likert scale. Only the emotional support scale was used in this study. The validity and reliability of the BSSS have been demonstrated in several studies (Schulz and Schwarzer, 2003). In the present study, Cronbach's alpha lay at 0.83 for the emotional support scale. Completion of the BSSS takes approximately 10 min.

Endocrine Measures

Saliva samples as well as capillary blood samples were collected in order to analyze endocrine parameters. The steroid hormones estradiol (E2), testosterone (T), progesterone (P4), and DHEA-S were assessed in saliva. Measurement of these steroids in saliva samples is well-established and reliable (for review, see Lewis, 2006; Gröschl, 2008). FSH, LH, and SHBG were determined in dried blood spot (DBS) samples. DBS sampling is an eligible and valid method to quantify these biomarkers, since highly sensitive and precise assays exist for the determination of FSH, LH, and SHBG (Worthman and Stallings, 1997; Edelman et al., 2007; McDade et al., 2007). Furthermore, DBS sampling is a viable and effective alternative to blood sampling by venipuncture, because it is less invasive and expensive, as well as easier to collect and store (Edelman et al., 2007).

Both saliva and capillary blood samples were obtained under standardized conditions at 8.00 am in the laboratory of the Department of Clinical Psychology and Psychotherapy of the University of Zurich. Prior to the sampling, the participants answered questions about incidents (e.g., infection or cold in the last week) and activities (e.g., sleep difficulties and subjective stress level at that moment) that could potentially have biased the current hormone concentrations.

Saliva Sampling

Saliva was collected with a SaliCap sampling tube of 2 mL capacity (IBL International GmbH, Hamburg, Germany). Participants were instructed to let the saliva flow to the base of the mouth before drooling into the tube using a polypropylene straw (passive drool method). Following the collection, saliva samples were stored at −20°C. The samples were thawed and centrifuged prior to biochemical analysis using IBL Saliva Immunoassays (IBL International GmbH, Hamburg, Germany). All saliva samples were analyzed in the biochemical laboratory of the Department of Clinical Psychology and Psychotherapy at the University of Zurich.

Dried Blood Spot Sampling

For the assessment of steroid hormone concentrations in dried blood, small capillary blood samples were drawn from participants' fingers. A sterile, disposable lancet (Accu-Chek® Safe-T-Pro Plus) was used for the finger prick on the middle finger of the non-dominant hand. Capillary blood drops were spotted onto standardized filter paper (Whatman® Protein Saver Cards, No. 903). The DBS samples were dried for 4 h and then frozen at −20°C. All DBS measures were analyzed in the Cytolab laboratory in Regensdorf, Switzerland.

Statistical Analyses

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 23). To compare pre- and postmenopausal women regarding their level of sexual functioning, a Mann-Whitney *U*-test (non-parametric alternative procedure to the independent samples *t*-test) was used in order to account for the heteroscedastic variances. In addition, we calculated a one-way ANOVA (Analysis of Variance) including age as a covariate, to examine whether differences in sexual functioning between pre- and postmenopausal women remain significant when adjusting for age. To examine the associations between main variables (age, psychosocial factors, and endocrine factors) and sexual functioning, bivariate and partial correlation analyses were calculated in a first step. To further determine the strength of these associations and to test possible predictors of sexual functioning, simple and multiple linear regression analyses were conducted in a second step. In addition, we examined the associations between the main variables and the dichotomous outcome of sexual functioning (absence vs. presence of healthy sexual functioning) according to the FSFI cutoff value of 26.55 proposed by Wiegel et al. (2005). For that reason, we first used bivariate correlation analyses to discover significant associations, and subsequently calculated logistic regression analyses to predict the odds of the dichotomous outcome. For all analyses testing the associations of psychosocial factors and sexual functioning, age and BMI were included as covariates. Additionally, systolic and diastolic blood pressure, medication intake and smoking status were statistically considered when testing the associations of steroid hormones and sexual functioning. Steroid hormone values were not normally distributed and were therefore log-transformed prior to the analyses. For all analyses including salivary steroids, 24 participants were excluded from the analyses due to gum bleeding (N = 2), an infection or injury in the mouth cavity (N = 3), or having had a cold (N = 19) in the days before the laboratory assessment. To adjust for multiple testing, we used the false discovery rate proposed by Benjamini and Hochberg (1995). The α-value of 0.05 was adjusted by (n+1)/2n, taking into account seven dependent variables per comparison (six

FSFI domain scores and total score). Therefore, an α-value of $p < 0.029$ was considered statistically significant. For all analyses testing the associations of the main variables with the dichotomous outcome of sexual functioning (absence vs. presence of healthy sexual functioning), the level of statistical significance was set at $\alpha = 0.05$.

RESULTS

Sample Characteristics

The final sample consisted of $N = 93$ healthy middle-aged and elderly women aged 40–73 years. **Table 1** provides the sociodemographic and health-related sample characteristics. The vast majority of participants were either pre- or postmenopausal. Regarding education, a vocational education or a college/university degree were most frequently reported. More than half of the sample was married, while the remaining participants were either single or in a common-law relationship. All of the participants were either hetero- or bisexual. About two thirds of the participants had their own children. The vast majority of the sample neither smoked nor took any medication.

TABLE 1 | Descriptive statistics of sociodemographic and health-related sample characteristics ($N = 93$).

Age, M ± SD	52.5 ± 8.5
Menopausal status, *n* (%)	
Premenopausal	45 (48.4)
Perimenopausal	6 (6.5)
Postmenopausal	42 (45.2)
Education, *n* (%)	
Vocational education	39 (41.9)
High school-leaving certificate	11 (11.9)
College/university degree	37 (39.8)
Other	6 (6.5)
Relationship status, *n* (%)	
Single	10 (10.8)
In a relationship	29 (31.2)
Married	54 (58.1)
Sexual orientation, *n* (%)	
Heterosexual	88 (94.6)
Bisexual	5 (5.4)
BMI (kg/m^2), M ± SD	22.71 ± 3.62
Blood pressure (mmHg), M ± SD	
Systolic blood pressure	121.77 ± 13.83
Diastolic blood pressure	79.58 ± 8.34
Smoking, *n* (%)	
Yes	12 (12.9)
No	81 (87.1)
Medication, *n* (%)	
Yes*	8 (8.6)
No	85 (91.4)

**Reported medication included antihypertensive drugs, cholesterol-lowering drugs, dietary supplements or other (acid blockers and gastric protection).*

TABLE 2 | Descriptive statistics of sexual function characteristics.

FSFI domain	*M ± SD*	*MIN, MAX*	*Range*
Desire	6.03 ± 1.36	2.00, 10.00	2–10
Arousal	15.96 ± 3.31	5.00, 20.00	0–20
Lubrication	16.82 ± 3.70	5.00, 20.00	0–20
Orgasm	12.45 ± 2.51	5.00, 15.00	0–15
Contentment	13.00 ± 2.28	5.00, 15.00	2–15
Pain	13.55 ± 2.45	2.00, 15.00	0–15
Total sexual functioning	29.05 ± 4.28	13.20, 36.00	2–36

Data are presented as mean (M) ± standard deviation (SD), minimum (MIN), maximum (MAX) scores, and range. Higher scores in the FSFI domains indicate better sexual functioning.

Sexual Function Characteristics

Descriptive statistics of sexual function characteristics (total FSFI and domain scores) are presented in **Table 2**. Higher scores in the FSFI domains indicate better sexual functioning. In our sample, 74 women (79.6%) scored above the cutoff value proposed by Wiegel et al. (2005), indicating healthy sexual functioning. As our sample comprised middle-aged and elderly women (Age: $M = 52.5$ years, $SD = 8.5$ years), we examined the impact of age and menopausal status on sexual functioning.

Age and Sexual Functioning

Age was negatively associated with arousal ($r = -0.302$, $p = 0.003$), lubrication ($r = -0.401$, $p < 0.001$), and overall sexual functioning ($r = -0.283$, $p = 0.006$). These negative associations were further confirmed using linear regression analyses. The results demonstrated that the lower the women's age, the higher their overall sexual functioning ($\beta = -0.267$, $p = 0.010$, adj. $R^2 = 0.61$), arousal ($\beta = -0.295$, $p = 0.004$, adj. $R^2 = 0.077$), and lubrication ($\beta = -0.393$, $p < 0.001$, adj. $R^2 = 0.145$). Furthermore, age was negatively associated with having healthy sexual functioning ($r = -0.318$, $p = 0.002$). Binary logistic regression analysis revealed that age [$b = -0.094$, Wald(1) = 8.312, $p = 0.004$] was a significant predictor of having healthy sexual functioning [Chi-Square(1) = 9.206, $p = 0.002$]. The odds ratio (OR) for age was 0.911 (95% CI 0.854−0.970), which means that for one added year of life, the relative probability to have healthy sexual functioning decreases 8.9% in the sample. Nagelkerke's R^2 was 0.148.

Menopausal Status and Sexual Functioning

Premenopausal ($N = 45$) and postmenopausal ($N = 42$) women were compared in terms of their sexual functioning. A Mann-Whitney U-test indicated that arousal was greater for premenopausal ($Mdn = 17.00$) than for postmenopausal ($Mdn = 16.00$) women ($U = 693.000$, $p = 0.031$). The two groups also differed significantly regarding lubrication ($U = 608.000$, $p = 0.003$), with premenopausal women having greater lubrication ($Mdn = 19.00$) than postmenopausal women ($Mdn = 16.00$). The significant group differences are illustrated in **Figure 3**.

In addition, we analyzed whether the differences in sexual functioning between pre- and postmenopausal women remained

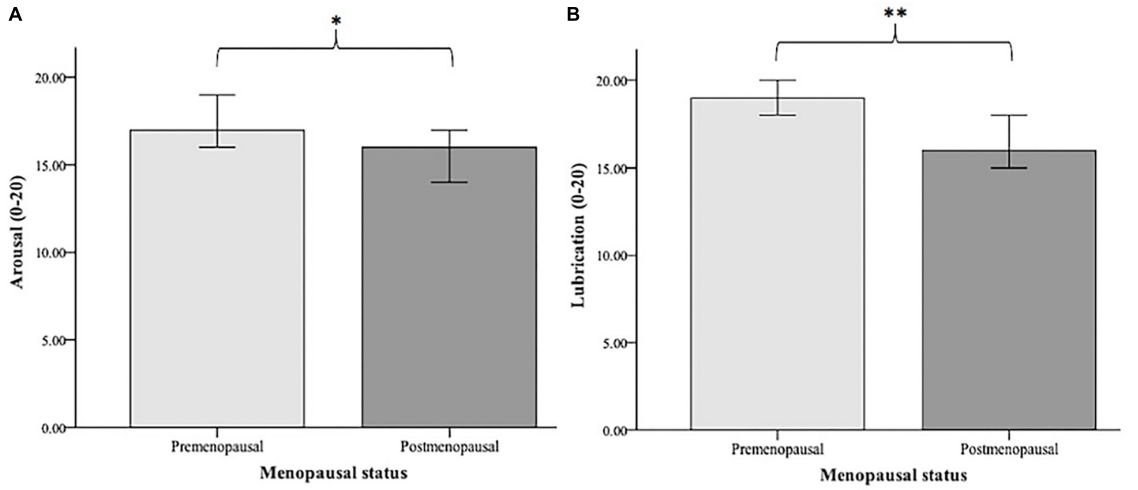

FIGURE 3 | Median values of arousal **(A)** and lubrication **(B)** in premenopausal (N = 45) vs. postmenopausal women (N = 42). Arousal and lubrication were measured using the Female Sexual Function Index (FSFI; Rosen et al., 2000). Significance level: *$p < 0.05$, **$p < 0.01$.

significant when adjusting for age. A one-way ANOVA including age as a covariate revealed that this was not the case.

Associations Between Psychosocial Factors and Sexual Functioning

To investigate the contribution of protective psychosocial factors to sexual functioning, we examined relationship-related factors (relationship satisfaction and emotional support) and psychological factors (self-esteem, optimism, extraversion, and life satisfaction) as possible predictors. In a first step, partial correlations between these psychosocial factors and sexual functioning were calculated, as illustrated in **Table 3**. The associations were further analyzed using regression analyses. Controlling for age and BMI, linear regression analyses revealed that overall sexual functioning (total FSFI score) was significantly predicted by optimism (β = 0.247, p = 0.018, adj. R^2 = 0.136), relationship satisfaction (β = 0.307, p = 0.005, adj. R^2 = 0.122), and emotional support (β = 0.246, p = 0.015, adj. R^2 = 0.110). Life satisfaction, self-esteem and extraversion were not significant predictors of overall sexual functioning (all $p > 0.029$).

The associations between the psychosocial factors and sexual functioning were further examined regarding the FSFI domains desire, arousal, lubrication, orgasm, contentment, and pain, using regression analyses. Optimism (β = 0.251, p = 0.015, adj. R^2 = 0.120) and relationship satisfaction (β = 0.289, p = 0.007, adj. R^2 = 0.126) each predicted arousal. Optimism was also a significant predictor of orgasm (β = 0.282, p = 0.009, adj. R^2 = 0.064). Sexual contentment was significantly predicted by the following psychosocial factors: relationship satisfaction (β = 0.484, $p < 0.001$, adj. R^2 = 0.239), emotional support (β = 0.370, $p < 0.001$, adj. R^2 = 0.172), self-esteem (β = 0.272, p = 0.008, adj. R^2 = 0.107), and life satisfaction (β = 0.330, $p < 0.001$, adj. R^2 = 0.141). The domain pain was only predicted by emotional support (β = 0.271, p = 0.009, adj. R^2 = 0.056). None of the tested psychosocial factors were predictive for the domains desire and lubrication (all $p > 0.029$).

In addition, we examined the associations between the psychosocial factors and the dichotomous outcome of sexual functioning (absence vs. presence of healthy sexual functioning). Emotional support was positively associated with having healthy sexual functioning (r = 0.253, p = 0.015), adjusted for age and BMI. Binary logistic regression analysis indicated that emotional support [b = 0.319, Wald(1) = 4.759, p = 0.029] was a significant predictor of healthy sexual functioning [Chi-Square(3) = 15.447, p = 0.001]. The OR for emotional support was 1.376 (95 % CI 1.033 – 1.833), which means that as emotional support increases one unit, the relative probability to have healthy sexual functioning increases 37.6%. Nagelkerke's R^2 was 0.240.

TABLE 3 | Summary of the partial correlation coefficients for the association of psychosocial factors with sexual functioning (N = 93).

	RAS	BSSS-ES	MSWS	LOT-R-O	BFI-K-E	SWLS
Desire	0.255	–0.012	0.053	0.222	0.105	0.080
Arousal	**0.297****	0.209	0.201	**0.253***	0.102	0.135
Lubrication	0.124	0.011	–0.019	0.007	–0.002	–0.011
Orgasm	0.041	0.246	0.186	**0.274****	0.168	0.081
Contentment	**0.488*****	**0.379*****	0.278**	0.223	0.218	**0.335****
Pain	0.227	**0.271****	0.243	0.125	0.158	0.116
Total sexual functioning	**0.314****	**0.254****	0.215	0.248*	0.169	0.163

*Covariates: age, BMI. RAS, relationship satisfaction; BSSS-ES, emotional support; MSWS, self-esteem; LOTR-O, optimism; BFI-K-E, extraversion; SWLS, life satisfaction. Significance level: *$p < 0.029$ (adjusted for multiple testing), **$p < 0.01$, ***$p < 0.001$.*

Associations Between Endocrine Factors and Sexual Functioning

As illustrated in **Table 4**, partial correlation analysis revealed that overall sexual functioning was not associated with any of

TABLE 4 | Summary of the partial correlation coefficients for the associations of steroid hormones with sexual functioning (N = 93 for blood steroids, N = 69 for salivary steroids).

	E2	P4	T	DHEA-S	FSH	LH	SHBG
Desire	0.132	0.006	0.083	−0.233	**−0.235**†	−0.165	0.084
Arousal	0.055	0.085	**0.256**†	−0.069	−0.077	−0.141	−0.070
Lubrication	0.060	0.023	0.060	−0.158	−0.145	−0.129	0.056
Orgasm	0.057	0.193	**0.316***	0.033	0.084	0.046	−0.047
Contentment	−0.035	−0.019	0.012	−0.207	−0.091	−0.114	−0.046
Pain	−0.001	0.103	0.068	−0.084	−0.131	−0.111	−0.006
Total sexual functioning	0.057	0.089	0.176	−0.153	−0.116	−0.136	−0.008

*Covariates: age, BMI, systolic and diastolic blood pressure, medication, smoking status. For the salivary steroids, N = 24 participants were excluded due to reports of a cold, infection, or gum bleeding before the laboratory assessment. E2, estradiol; P4, progesterone; T, testosterone; DHEA-S, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin. Significance level: *$p < 0.029$ (adjusted for multiple testing), †$p < 0.05$.*

the tested steroid hormones (adjusted for age, BMI, systolic and diastolic blood pressure, medication, and smoking status). When investigating the single FSFI domains and steroid hormones, testosterone was positively associated with orgasm. Regression analyses further confirmed that testosterone was a significant predictor of orgasm ($\beta = 0.329$, $p = 0.012$, adj. $R^2 = 0.089$).

Additionally, we examined the associations between the endocrine factors and the dichotomous outcome of sexual functioning (absence vs. presence of healthy sexual functioning). Partial correlation analysis (including the covariates) showed no significant associations with the tested steroid hormones. Therefore, the endocrine factors were not entered into a binary logistic regression model.

DISCUSSION

This study investigated psychobiological predictors of sexual functioning in a healthy sample of middle-aged and elderly females. For this purpose, we examined the associations of age, menopausal status, protective psychological and interpersonal factors, as well as endocrine factors with female sexual functioning and its various components. First, we found that age and menopausal status were negatively associated with the level of sexual functioning in the sample. Second, psychosocial factors emerged as the key predictors of sexual functioning. To our knowledge, this is the first study to test and demonstrate the importance of optimism and emotional support for healthy women's sexual functioning in midlife and older age. Third, we found that sex hormones were not related to sexual functioning in this healthy sample.

As our sample comprised middle-aged and elderly women (40–73 years), it was necessary to take into account age and menopausal status for the investigation of sexual functioning. Negative effects of age on female sexual functioning are frequently reported in the literature and also reflected in the prevalence rates of sexual dysfunction across different age ranges (e.g., Nicolosi et al., 2004). However, the findings differ in terms of which aspects of sexual functioning are affected by age. In the present study, younger age was associated with higher levels of arousal, lubrication, and overall sexual functioning. This is partly consistent with the existing literature. Most of the previous studies investigated the effects of age on sexual desire, interest, and frequency of sexual activity (reviewed in Hayes and Dennerstein, 2005), with the majority of findings revealing a decrease in sexual desire and interest (e.g., DeLamater and Sill, 2005). Research on the effects of age on arousal in females is limited (Hayes and Dennerstein, 2005). The available studies have reported both a decline in arousal with increasing age (Lee et al., 2016) as well as no changes (Cain et al., 2003). The negative relationship between age and lubrication can be biologically explained by age-associated changes in the urogenital system. As women age, vaginal symptoms such as vaginal dryness emerge more often due to the reduced production of mucus from the glands of the vaginal wall as a sign of vaginal atrophy (Samsioe, 1998). However, these age-associated physiological changes are confounded by the hormonal effects of the menopausal transition phase, and therefore need to be carefully disentangled from the effect of menopausal status on sexual functioning (Dennerstein et al., 2001). In our study, however, we found that menopausal status was only related to sexual functioning independently of age. Finally, the high correlation between sexual arousal and lubrication (as one of the main physiological underpinnings of arousal) may explain why both of these aspects are associated with age.

Regarding the relevance of menopausal status for sexual functioning, we found that pre- and postmenopausal women only differed in their level of sexual arousal and lubrication. Overall sexual functioning differed only at a trend level ($p < 0.10$). These findings are only partly consistent with the literature. According to various longitudinal studies, the transition to menopause is related to some, but not necessarily all, aspects of sexual functioning. For example, Avis et al. (2000) found a lower level of sexual desire and arousal with advancing menopausal status, while other aspects of sexual functioning remained unrelated to menopausal status. In another study, lubrication emerged as the domain of sexual functioning with the most pronounced decline during the transition to menopause (Gracia et al., 2007). Other findings revealed that pain during sexual intercourse increases, while only sexual desire

decreases across the menopausal transition (Avis et al., 2009). These inconsistencies might be due to methodological issues concerning the differing measurement of sexual functioning or the dependency of sexual functioning on other factors besides age and menopausal status, such as psychosocial factors in midlife and older age.

In the present study, we found several psychosocial predictors of sexual functioning. A high level of relationship satisfaction and emotional support were associated with better overall sexual functioning in this healthy sample. The importance of relationship-related factors for sexual health in midlife and older age has already been discussed in the literature (Brotto et al., 2016). In line with previous research findings, relationship satisfaction seems to be among the most important determinants of female sexual functioning (Dennerstein et al., 2005; Thomas and Thurston, 2016). Our results provide additional evidence for the importance of relationship satisfaction, because it was a predictor not only of sexual contentment, but also of arousal and overall sexual functioning. The partner plays a key role for a woman's satisfying sexuality, since the partner's sexual functioning may have an impact on the females' sexual functioning (DeLamater and Karraker, 2009). There is evidence showing a strong correlation between females' and males' sexual functioning (Yeoh et al., 2014). Therefore, one's own sexual wellbeing may not only be dependent on the satisfaction with the relationship, but also on the partner's sexual wellbeing. However, social support, and in particular emotional support, had not been previously investigated. Interestingly, our study showed that emotional support was a predictor of overall sexual functioning as well as of particular aspects, namely sexual contentment and pain. Moreover, to our knowledge, the present study was the first to examine the role of optimism for the sexual functioning of healthy middle-aged and elderly females. Optimism is defined as a personality disposition characterized by generalized positive outcome expectancies, and has been shown to be positively associated with physical health and mental well-being (Carver and Scheier, 2014). We found that a higher level of optimism was related to a higher level of overall sexual functioning, arousal, and orgasm. Furthermore, life satisfaction and self-esteem were associated with sexual contentment in our study, but not with overall sexual functioning. We assume that life satisfaction may be specifically connected to satisfaction in other areas of life, such as sexuality, which would explain the association with sexual contentment. Self-esteem represents a central dimension of the self-concept, which has been shown to have health-promoting effects on a variety of outcomes regarding, for example, emotion, cognition, and motivation (Goodson et al., 2006). So far, the relationship between self-esteem and sexual functioning has not been investigated in detail. Our results are partly in line with a study showing that collegiate women with higher self-esteem reported greater sexual contentment and orgasmic response (Rehbein-Narvaez et al., 2006). However, there is a lack of studies including women in middle and older age. Finally, it is interesting to note that none of the tested psychosocial factors in this study were related to sexual desire or lubrication. Further research is needed to confirm our findings and to examine psychosocial factors associated with desire and lubrication.

As part of the biopsychosocial approach, we also examined the role of endocrine factors in the sexual functioning of healthy females. None of the tested endocrine parameters (E2, T, DHEAS, P, FSH, LH, and SHBG) were linked to overall sexual functioning. This argues against the assumption that sex steroids are important for female sexual functioning due to their age- and menopause-associated fluctuations (Clayton and Harsh, 2016). From that perspective, it is surprising that sex steroids were not associated with sexual functioning in our sample of pre- and postmenopausal women. From yet another perspective, however, our findings are in line with the majority of current studies and overviews pointing to weak or no correlations between sex steroids and female sexual functioning (Meston and Frohlich, 2000; Dennerstein et al., 2005; Wierman et al., 2010; Randolph et al., 2015; Worsley et al., 2016). An explanation for the observed results in this study may lie in the rather low variability across our study sample. All of our participants reported to be physically and mentally healthy, did not take any hormonal agents and reported rather low levels of menopausal symptoms, which may indicate an adaptive adjustment to hormonal fluctuations. Taking this into account, we can speculate that endocrine factors might only be determinants of sexual dysfunction rather than sexual function. Additional research is required to test this hypothesis and to further examine the contribution of sex steroids to women's overall sexual functioning in midlife and older age. Another potential explanation for the non-significant findings may concern the validity of the saliva samples for the hormones measured in our elderly sample. Although the assessment of E2 in saliva samples is valid and salivary E2 is considered as an adequate estimate of serum E2 (Fiers et al., 2017), there is data suggesting that salivary E2 may only predict serum E2 among postmenopausal women who use estrogen therapy (Tivis et al., 2005). According to the authors, E2 levels may be too low among postmenopausal women who do not use estrogen therapy and therefore cannot be adequately detected via saliva samples.

However, we found that a specific aspect of sexual functioning, namely orgasm, was positively associated with the level of testosterone. Testosterone is the sex steroid which is assumed to primarily influence sexual desire and motivation (Bachmann and Leiblum, 2004; Wåhlin-Jacobsen et al., 2015). Evidence for the importance of testosterone in female sexual functioning mainly stems from studies demonstrating that testosterone replacement therapy improves sexual functioning (Shifren et al., 2000; Goldstat et al., 2003). Pharmacological agents such as testosterone or Flibanserin, a 5-hydroxytryptamine 5-HT1A agonist and 5-HT2A antagonist, are frequently reviewed as treatment options for poor sexual desire and low sexual functioning in mostly premenopausal women (Roslan et al., 2019; Simon et al., 2019). However, their efficacy and safety is the subject of controversial discussion in the current literature.

The present study has several strengths. First, it is one of the few studies to investigate exclusively healthy females in midlife and older age. Strict inclusion criteria ensured

that the examined women were physically and mentally healthy, and did not take any hormone replacement or psychotropic medication. Second, we applied a biopsychosocial health approach and therefore included various perspectives, examining biological, psychological, and interpersonal factors of the complex phenomenon of female sexual functioning. Furthermore, we focused on positive and protective factors in relation to optimal sexual functioning, which have rarely been investigated so far. This notion is in line with positive health concepts that stress the importance of strengths and resources rather than risks and pathology (Graugaard, 2017).

Some limitations of the present study should also be acknowledged. The Women 40+ Healthy Aging Study has a cross-sectional design, in which psychosocial and hormonal data were gathered at only one time point. Therefore, it was not possible to infer causality of the tested associations. Further, larger studies are required to confirm our results. Additionally, future longitudinal examinations are needed to examine changes in sexual health and functioning over time and the associated psychobiological factors. In particular, multiple measurements of endocrine parameters over time would be preferable in order to account for intraindividual hormonal fluctuation, which is especially relevant in the investigation of sex hormones in pre- and perimenopausal women. Furthermore, the use of saliva and DBS samples to measure sex hormones in middle-aged and elderly women needs to be carefully validated in future studies. Although the quantification of the mentioned hormones in saliva and DBS samples is a reliable approach and has several advantages, it may also be inferior to measuring serum levels in some samples or subgroups like postmenopausal women (see Tivis et al., 2005). Another limitation refers to the assessment of sexual functioning and the associated sample size reduction in our study. The FSFI (Rosen et al., 2000) is considered to be the gold standard in the assessment of female sexual functioning and is broadly used in clinical and epidemiological research. Due to its dependence on sexual activity, women with no engagement in sexual activity over the last 4 weeks had to be excluded from the study. According to Rosen et al. (2000), the FSFI should only be applied to women who have had sexual activity during the measurement period. From a total of 130 women participating in the Women 40+ Healthy Aging Study, 13 participants reported not having been sexually active in this time frame. Unfortunately, 14 more study participants showed inconsistencies when reporting whether they engaged in sexual activity in the last month and therefore also had to be excluded. For most of the FSFI items, it was possible to select *"no sexual activity"* as a response option, leading to within-subject inconsistencies across different items in terms of the reported sexual (in-)activity in these women. We can only speculate about possible reasons for this response behavior. We did not find any differences between the women who responded inconsistently and the rest of the sample with regard to health variables or relationship status. However, it is interesting to note that for several of the women concerned, sexual activity was reported primarily at the beginning of the questionnaire and sexual inactivity toward the end. This may be due to the occurrence of partner-related questions toward the end (e.g., item number 14: "Over the past 4 weeks, how satisfied have you been with the amount of emotional closeness during sexual activity between you and your partner?"). Possible explanations are that some of the women did not want to answer sexual partner-related questions or that the instructions of the questionnaire regarding the definition of sexual activity had not been understood correctly. Alternatively, the inconsistent response behavior may be due to a potential unwillingness to answer some of the questions for reasons of privacy or non-disclosure. Thus, our results are important in that they demonstrate that sexuality is still a rather taboo topic that may be regarded with shame in some middle-aged and older women.

Some practical implications may be derived from our findings. The relevance of psychosocial factors such as social support or self-esteem for female sexuality in midlife and older age suggests potential starting points from which to foster sexual functioning. Psychotherapeutic strategies targeting the social context, self-concept or aspects of personality may help to optimize women's sexual functioning in aging. Furthermore, in clinical practice, the complete assessment of biopsychosocial aspects contributing to sexual functioning is necessary in order to guide the management of middle-aged and older adult's sexual health.

In conclusion, this study showed that sexual functioning in healthy middle-aged and older women is highly dependent on psychosocial aspects that are health-promoting or related to well-being, such as interpersonal factors and protective psychological traits. Endocrine factors seem to be of secondary importance in this regard. To further investigate female sexual health and its associated psychobiological underpinnings, a focus on sexual function rather than dysfunction may be the better approach.

AUTHOR CONTRIBUTIONS

All authors conceived and planned the study, contributed to the interpretation of the results, provided critical feedback, and helped shape the research, analysis and manuscript. LM took the lead in writing the manuscript.

FUNDING

The study project Women's Health 40+ was fully funded by the University Research Priority Program (URPP) *Dynamics of Healthy Aging* of the University of Zurich.

ACKNOWLEDGMENTS

We would like to thank all women who participated in our study. We also wish to express our gratitude to Dr. Firouzeh Farahmand and M.Sc. Elena Gardini for performing the analyses of salivary hormones for this study.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 22 May 2019
doi: 10.3389/fpsyg.2019.01101

Subjective and Oxytocinergic Responses to Mindfulness Are Associated With Subjective and Oxytocinergic Responses to Sexual Arousal

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Mindfulness – the ability to pay attention, on purpose, without judgment, and in the present moment – has consistently been shown to enhance women's sexual arousal. As a first step toward understanding potential neuroendocrine underpinnings of mindfulness and sexual arousal, we examined whether individual differences in subjective and neuroendocrine (i.e., oxytocin) responses to mindful breathing were associated with individual differences in subjective and neuroendocrine responses to sexual arousal. To achieve this aim, 61 lesbian, bisexual, and heterosexual women completed a questionnaire assessing dispositional mindfulness, underwent an arousal task while continuously rating their sexual arousal and a mindful breathing task, after which participants reported on their ability to detect attentional shifts, and provided salivary samples after each assessment. Results indicated that women who were quicker to detect attentional shifts and women who reported greater sexual arousability reported larger changes (decreases) in oxytocin in response to mindful breathing and were the only women to report increases in oxytocin in response to the sexual arousal induction. Results further indicated that individuals who report greater subjective responsiveness to mindfulness and sexual arousal appear to have an oxytocinergic system that is also more responsive to *both* arousal and to mindfulness. These results make a significant contribution to our understanding of the role of attentional processes in sexual arousal, and warrant future examination of oxytocin as a potential neuroendocrine mechanism underlying the link between mindfulness and sexual arousal.

Keywords: oxytocin, sexual arousal, mindfulness, attentional shifts, women, sexual health

OPEN ACCESS

Edited by:
Beate Ditzen, University Hospital Heidelberg, Germany

Reviewed by:
Adam Safron, Northwestern University, United States
Erin Walsh, The University of North Carolina at Chapel Hill, United States

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Specialty section:
This article was submitted to Health Psychology, a section of the journal Frontiers in Psychology

Received: *05 November 2018*
Accepted: *26 April 2019*
Published: *22 May 2019*

Citation:
Dickenson JA, Alley J and Diamond LM (2019) Subjective and Oxytocinergic Responses to Mindfulness Are Associated With Subjective and Oxytocinergic Responses to Sexual Arousal. Front. Psychol. 10:1101. doi: 10.3389/fpsyg.2019.01101

INTRODUCTION

Female sexual arousal depends on one's ability to attend to physical sensations and emotional experiences (Janssen et al., 2000; Spiering and Everaerd, 2007). Robust research has demonstrated that a specific form of attention – mindfulness – enhances sexual arousal. However, not all people are equally sensitive to mindfulness nor are all women equally *arousable* (i.e., the degree and ease

in which women subjectively and nuerobiologically experience sexual arousal in response to sexual contexts). Yet, we have limited understanding of how such individual differences in arousability may be related to individual differences in *responsiveness to mindfulness*, involving the degree to which women subjectively respond (e.g., ease of attention, greater relaxation response) and the degree of neuroendocrine release in response to mindfulness inductions. One possibility is that those who are more responsive to mindfulness are also more arousable due to an increased biological sensitivity to contextual influences, marked by a neuroendocrine system with greater potential to benefit from mindfulness during mindful breathing and for greater arousability within a sexual context. Because oxytocin is involved in sexual arousal, enhances salience of social stimuli, and buffers the stress response, the oxytocinergic system may provide insight into why, how, or for whom mindfulness impacts arousal. The current study investigates whether lesbian, bisexual, and heterosexual women who are more responsive to mindfulness are also more arousable (responsiveness of the arousal system) and show larger changes in oxytocin to both a sexual arousal induction and a mindful attention induction.

Mindfulness – "to pay attention in a particular way: on purpose, in the present moment, and non-judgmentally" (Kabat-Zinn, 1990) – has received extensive investigation for its powerful stress-buffering effects across various mental health concerns (Grossman et al., 2004; Hofmann et al., 2010; Britton et al., 2012) and improving physical health outcomes (Kabat-Zinn et al., 1985; Davidson et al., 2003; Ludwig and Kabat-Zinn, 2008). Mindfulness involves intentionally bringing attention to one's present moment thoughts, emotions, and sensations, with a spirit of curiosity, gentleness, and kindness. Training of mindfulness often begins with learning a mindful breathing exercise, in which practitioners gently focus their attention to the moment-to-moment physical sensations of breathing, allowing whatever physical sensations to be there, without judgment or attempting to control or change their experience in any way. Practitioners are taught that whenever their mind wanders away from their breath presents an opportunity to become mindful (Stahl and Goldstein, 2010). Training individuals to detect these attentional shifts (Doll et al., 2015) enhances attentional control (Dickenson et al., 2012; Hasenkamp et al., 2012; Kerr et al., 2013; Malinowski, 2013). By attending to, recognize, and label their present moment emotions, thoughts, and sensations, mindfulness practice also cultivates a compassionate relationship to one's experience and promotes self-acceptance (Hollis-Walker and Colosimo, 2011; Keng et al., 2012).

Previous research has consistently linked trait mindfulness with various aspects of sexuality (Brotto et al., 2008; Adam et al., 2015a; Khaddouma et al., 2015; Arora and Brotto, 2017; Greer, 2017). Women with higher overall levels of trait mindfulness report greater sexual arousability (Mayland, 2005) and specific facets of trait mindfulness have been associated with various indices of sexuality. Women who report a greater tendency toward observing their internal thoughts, emotions, and sensations, notice their internal experiences without judgment (not labeling them as good or bad), and remain in present moment awareness (rather than behaving automatically) report greater sexual and relationship satisfaction (Khaddouma et al., 2015) and less sexual distress (Adam et al., 2015b). Additionally, present moment awareness, noticing internal experiences without judgment, and describing internal experiences with words reduce cognitive distractions that interfere with arousal and predict greater sexual functioning (Dunkley et al., 2015). These studies suggest that reporting greater tendencies to observe one's experience in the present moment, without judgment contribute to greater overall levels of sexual arousal.

Mindfulness training also enhances arousal among women with various levels of arousability. For example, training mindful attention has proven effective in enhancing interoceptive awareness to sexual sensations and subjective sexual arousal among healthy women (Silverstein et al., 2011) and among women with sexual interest and arousal disorders (Brotto et al., 2016). Moreover, research has demonstrated that mindfulness training can have different impacts on different people (Arora and Brotto, 2017), suggesting that individual differences in women's *responsiveness* to mindfulness practice may correspond to individual differences in women's responsiveness to sexual contexts, or arousability.

Mindfulness may augment arousal via enhancing the salience of interoceptive stimuli in response to external sexual cues. For example, mindfulness training appears to have little effect on increasing physiological arousal, but increases the ability to attend to whatever physical sexual sensations are present, heightening the salience of interoceptive cues resulting in greater subjective arousal (Brotto et al., 2016). Additionally, improvements in sexual functioning resulting from mindfulness-based interventions are mediated by changes in trait mindfulness and overall levels of self-compassion (Paterson et al., 2017). The abovementioned research indicates that trait mindfulness is associated with greater sexual arousal, mindfulness training augments sexual arousability, and trait mindfulness is associated with greater responsiveness to mindfulness training. Such research indicates that the arousal-enhancing effects of mindfulness might stem from engendering a non-judgmental approach to observing one's present moment thoughts, emotions, and sensations in response to internal and external cues. To date, a dearth of research has examined potential biological pathways that explain the links between trait mindfulness, responsiveness to mindfulness inductions, and sexual arousability. Oxytocin provides an ideal avenue for investigating links between individual differences in mindfulness (trait mindfulness and responsiveness to mindfulness practice) and individual differences in arousability, given its role in sexual behavior, stress reduction, and enhancing the salience of internal and external social stimuli.

Oxytocin is best known for the role that it plays in romantic and parental bonding (Carter, 1992). However, oxytocin also increases during sexual arousal and peaks during orgasm (Carmichael et al., 1987; Blaicher et al., 1999). Additionally, oxytocin is associated with greater subjective sexual arousal (Burri et al., 2008), contributes to the rewarding nature of sexual interactions (Veening et al., 2015), and integrates social attention, emotional feelings, and the functions of the autonomic

system. Within its social function, oxytocin increases the salience of external social cues and interoceptive responses to social cues. Specifically, oxytocin affects the evaluation of emotions during early stages of information processing (Parr et al., 2013), modulates attentional orienting to social cues (Leknes et al., 2012), and buffers the stress response to social challenges (Heinrichs et al., 2003) by regulating cortisol responses (Devries et al., 2003), which allows individuals to approach challenges rather than avoid threats (Harari-Dahan and Bernstein, 2014). Moreover, oxytocin been linked to achieving greater emotional benefits from socially oriented forms of meditation practices, such as loving kindness (Isgett et al., 2016; Van Cappellen et al., 2016). Although the non-social effects of oxytocin has received much less attention, research has demonstrated that oxytocin facilitates the salience of personally relevant and emotional stimuli in non-social contexts (Harari-Dahan and Bernstein, 2014). For example, oxytocin facilitates a specific form of present-moment awareness – the feeling of becoming fully immersed in one's experience (Piper et al., 2015; Pohling and Diessner, 2016). Because oxytocin facilitates salience of personally relevant and emotional stimuli, perhaps oxytocin facilitates non-judgmental approach to observing one's present moment thoughts, emotions, and sensations in response to sexual cues and in response to mindfulness practice.

Current Study

The aim of the current study was to examine whether oxytocin represents a potential biological pathway that elucidates the links between individual differences in trait mindfulness, responsiveness to mindfulness practice, and sexual arousability. To achieve this aim, 61 lesbian, bisexual, and heterosexual women reported their levels of trait mindfulness, completed a sexual arousal induction followed by a mindful breathing induction, and provided salivary oxytocin and cortisol samples. We used multilevel random coefficient modeling to examine the rate of change in oxytocin (measured after a baseline period, following the arousal and mindful breathing tasks, and after an unstructured recovery period) to answer the following objectives:

(1) *Does oxytocin change in response to a sexual arousal induction and a mindful breathing induction?* We predicted that oxytocin would increase in response to the sexual arousal and mindful breathing induction, relative to baseline.
(2) *Do the specific facets of trait mindfulness modulate the change in oxytocin in response to a sexual arousal induction and a mindful breathing induction?* We predicted that women who report a greater tendency to observe one's thoughts, emotions, and sensations, notice their internal experiences without judgment, and remain in present moment awareness would show larger changes in oxytocin in response to the sexual arousal and mindful breathing inductions, relative to baseline.
(3) *Do subjective responsiveness to the sexual arousal and mindfulness inductions modulate change in oxytocin and do women who are more subjectively responsive have an oxytocinergic system that is broadly more responsive to various contexts* (i.e., *greater change in oxytocin irrespective of the type of induction*)*?* We predicted that women who report greater subjective responsiveness to the sexual arousal and mindfulness breathing inductions (i.e., greater arousability and quicker ability to detect attentional shifts) would report a more responsive oxytocinergic system irrespective of the type of induction, such that they would report larger changes in oxytocin in response to *both* the sexual arousal and the mindful breathing inductions, relative to baseline.
(4) *Are women who are subjectively and biologically more responsive to a mindfulness induction are also subjectively and biologically more sexually arousable?* We predicted that women who were more subjectively responsive to a mindful breathing induction would also report greater sexual arousability during a sexual arousal induction. We predicted that women who show larger changes in oxytocin in response to the sexual arousal induction also show larger changes in oxytocin in response to the mindful breathing induction.

MATERIALS AND METHODS

Participants

Participants included women between the ages of 20 and 35 (mean age = 27.2). In all, 35% of the women identified as heterosexual, 44% as bisexual, and 21% as lesbian. In all, 91.1% of the women were White, 12.2% identified as Latina, 4.4% identified as Asian/Pacific Islander, 2.2% identified as Other Race, and 1.1% identified as African American. Participants were recruited through Facebook ads that described the study as an investigation of sexuality and stress hormones, and was approved by the University of Utah Institutional Review Board. Because two participants had incomplete data, they were dropped from analysis, resulting in a total of 61 participants. All participants had normal, natural menstrual cycle and were not taking any medications that could affected their sexual functioning (e.g., antidepressants). To control for digestive process on neuroendocrine release and time of day, participants were instructed to abstain from any food or caffeine intake approximately 2 h before their visit, which occurred between 3 pm and 7 pm.

Procedures

Approximately 10 days after participants' began menstruating, participants arrived at the laboratory to provide written informed consent and answer questions pertaining to their sexual history, current sexual attractions and experiences, and demographics. Women were then escorted to a private room to participate in the arousal induction task, where they underwent a 15 min baseline period. During the first 5 min, they sat quietly. During the second 5 min, they rated their liking of a set of landscape photographs, in order to engage their attention in a restful pleasant task (Jennings et al., 1992). During the remainder of the baseline period, they paced their breathing slowly in response to a timer (4 s of

inhalation, 4 s of exhalation). Women then provided oxytocin and cortisol salivary samples.

Next, women listened to a series of neutral and erotic stories, which have been shown to reliably elicit sexual arousal among women (Chivers and Timmers, 2012). The stories varied by the nature of the interaction as well as the gender of the person in the story described. For example, each participant heard various stories that were depicted interacting with a man or a woman in sexual or non-sexual way (Chivers and Timmers, 2012). Each story was read to the participant through headphones, lasted about a minute and a half, and contained explicit language and situations. There was a one-min recovery period between each story, so that the total amount of time listening to the stories was 18 min. Throughout the duration of this task, women provided continuous ratings of their sexual arousal. Following this task, women provided a second salivary oxytocin sample, marking the index of oxytocin responsivity to sexual arousal.

Next, women underwent a focused breathing induction, in which participants were instructed to mindfully attend to their breath using a script adapted from previous research (Arch and Craske, 2006; Dickenson et al., 2012). Participants were instructed to attend to the moment-by-moment physical sensations of their breath and whenever their mind wanders to congratulate yourself each time on reconnecting with your experience in the moment and gently escorting the attention back to the breath. Following this task, women completed a series of questions about their experience during the mindful breathing task and provided the third salivary sample, which marks oxytocin responsivity to mindful breathing. They were then escorted back to the open room where spent an additional 15 min sitting quietly and filling out additional questionnaires and were oriented to additional aspects of the study. Participants provided their final salivary sample, which marks the "recovery" period. Hence, each of the four saliva samples were collected 15–20 min apart.

Participants additionally completed a secondary laboratory assessment that was nearly identical to the first session with one exception. After the baseline period, women underwent a modified version of the Trier Social Stress Test (Kirschbaum et al., 1993), including the performance of a speech and the completion of a difficult serial subtraction task in the presence of an experimenter. This task is not discussed in the current study, other than serving to compare oxytocin responses during mindful breathing to explore potential order effects. After this session, participants were debriefed and reimbursed for their time. This study was approved by University of Utah Institutional Review Board.

Task Based Measures

Subjective Arousability

Participants recorded their ratings for arousal continuously throughout the arousal task. In order to obtain a measure of *responsiveness* to the arousal induction (or arousability), we used peak ratings from the erotic stories. Given that we included lesbian, bisexual, and heterosexual women, we included erotic stories that depicted men and stories that depicted women. We computed women's self-reported peak ratings for each of the erotic stories featuring their self-reported preferred gender (inferred from self-report of their attractions) and for each of the erotic stories featuring their non-preferred gender.

Subjective Responsiveness to Mindful Breathing

Following the mindful breathing task, participants rated their extent of agreement with a series of statements including "I was restless and bored during this experience," "I felt calm and relaxed," and "It took me a while to notice that my mind had wandered." Because the former questions were highly correlated, we aggregated these scores into a composite measure of relaxation response to mindful breathing. Given that being able to detect when one's mind has wandered is a central component of mindfulness training, we examined the mind wandering question as a single-item measure indicative of one's ability to detect attentional shifts. This item was reverse coded such that higher scores indicated being quicker to detect attentional shifts.

Trait Mindfulness

To limit participant burden of completing myriad questionnaires, we used a modified version of the five facet mindfulness questionnaire (FFMQ), which assessed 25-items of the FFMQ, corresponding to five related dimensions of trait mindfulness. All items of the FFMQ are rated on a 5-point Likert scale, ranging from 1 (*never or very rarely true*) to 5 (*very often or always true*). All subscales corresponding to the dimensions had good reliability. The observing subscale ($\alpha = 0.78$) indicates the degree of observing (attending or noticing) internal (e.g., bodily sensations, thoughts, and emotions) and external sensations (e.g., smells, sights, and sounds). The Describing subscale ($\alpha = 0.84$) reflects the ability to express in words one's own experience. The acting with awareness ($\alpha = 0.85$) subscale reflects attending to one's present moment activity, rather than behaving automatically, without much thought or attention and is the subscale that mirrors constructs closest to other trait mindfulness scales (e.g., MAAS; Carlson and Brown, 2005). The non-judging of Inner Experience ($\alpha = 0.89$) subscale reflects an ability to accept thoughts and emotions without judging them (i.e., evaluating as good or bad). The non-reactivity to Inner Experience ($\alpha = 0.77$) subscale reflects the ability to detach from one's inner experience (i.e., thoughts and emotions) and allowing thoughts and emotions to come and go without clinging to or avoiding them. Because these five facets are related, but distinct constructs, separate scores for each subscale were calculated to be entered into the analytic models (non-significant subscale scores were removed from statistical models).

Endocrine Measures

For the collection of salivary oxytocin data, participants collected approximately 1 mL of saliva in their mouths and released the saliva into a glass centrifuge tube using the "passive drool" technique. Samples were immediately frozen at −25°C until shipped on dry ice to the University of North Carolina, Chapel Hill. The oxytocin enzyme-immunoabsorbance (EIA) method used to assay salivary oxytocin is identical to that reported previously (Holt-Lunstad et al., 2008). Salivary oxytocin

levels were measured using the oxytocin EIA (Enzo Life Sciences, Farmingdale, NY, United States). After correcting for concentration produced by extraction, the lower limit of sensitivity was 1.5 pg/mL, with intra- and inter-assay variations of 4.8 and 8%.

The current study also controlled for cortisol levels, given that oxytocin has been linked to cortisol. The specific link between cortisol and oxytocin are reported elsewhere (Alley et al., 2019) and, thus, were not the focus of the current study. Salivary cortisol samples were taken using salivettes (Sarstedt, Germany), consisting of a plastic tube with a cotton insert. The participant was instructed to lightly chew on the insert to thoroughly soak it with their saliva. All samples were kept frozen at −25°C until being shipped on dry ice to be assayed by the laboratory of Dr. Kirschbaum at the Technical University of Dresden, which uses a time-resolved immunoassay with fluorometric end point detection (see Dressendorfer et al., 1992) with intra- and inter-assay precision of 3.0 and 4.2%. In all, 7% of cortisol samples were either missing or could not be assayed, and follow-up analyses detected no systematic patterns of missing samples (i.e., no correlations with other study variables).

Analytic Strategy

Analyses were conducted with multilevel random coefficient modeling (MRCM, employed with WHLM; Bryk and Raudenbusch, 1992), to represent the nested nature of the data, in which lower level units (oxytocin) vary within persons. Specifically, piecewise linear growth modeling was conducted to examine changes in oxytocin levels across each time period, which allows for the division of growth trajectories into separate linear components and is a common approach to examine responsivity and recovery processes. The first linear component (responsivity), coded 0 as baseline and 1 for the remaining 3 time points, represents the change between baseline, and arousal/stress responses. The second linear component, coded 0 for baseline and for the arousal/stress inductions and 1 for the remaining tasks, represents the rate of change in oxytocin during the mindful breathing induction. The third linear component, coded 0 for the first three samples and 1 for the recovery sample, represents the rate of change in oxytocin at the end of the visit (recovery). Because of positive skew, both oxytocin and cortisol were log-transformed for analysis. The Level 1 model has the following structure:

$$\text{Oxytocin}_{\text{time t, participant i}} = \pi_{0i} + \pi_{1i}(\text{Sexual Arousal}) + \pi_{2i}(\text{Mindful Breathing}) + \pi_{3i}(\text{Recovery}) + \pi_{4i}(\text{Cortisol}) + e_{ti}$$

where π_{0i} represents baseline oxytocin, π_{1i}, π_{2i}, and π_{3i} represent the rate of linear change in oxytocin across the sexual arousal induction, rate of change during mindful breathing, and rate of change following a recovery period, and e_{ti} represents the within-individual error in participant *i*'s oxytocin that cannot be accounted for by cortisol levels (π_{4i}), baseline oxytocin (π_{0i}), or by change over time (π_{1i}, π_{2i}, and π_{3i}).

These Level 1 associations were then modeled as a function of an intercept and a random effect at Level 2 to obtain overall estimates of baseline and rate of change in oxytocin, controlling for cortisol levels. Non-significant random effects were dropped from this unconditional model. Next, we modeled the Level 1 associations as a function of Level 2 variables to examine whether the size and/or direction of these effects varies from woman to woman.

The Level 2 model calculated whether women who showed greater subjective sexual arousal, greater subjective responses to mindful breathing, and higher levels of trait mindfulness showed differences in their oxytocin levels at baseline, in response to the sexual arousal induction, and in response to mindful breathing. Level 2 variables were grand centered, such that the Level 2 intercepts represent the average rate of change in oxytocin at average (sample mean) levels of peak sexual arousal, subjective responses to mindful breathing, and trait mindfulness. To achieve the most parsimonious model to test our hypotheses, oxytocin recovery and the link between oxytocin and cortisol were modeled as a function of the intercept and error. As well, variables (representing level 2 moderators of level 1 associations) that were non-significant were dropped from the model, resulting in the final level 2 model that predicted rate of change in oxytocin from trait mindfulness (present moment awareness and noticing internal experiences without judgment), detecting attentional shifts, and sexual arousability toward the preferred sex. Significant Level 2 effects were followed up with simple slopes at 25th and 75th percentiles around the mean of each continuous moderator. Additionally, because previous research has found that the facets of trait mindfulness may interact to predict outcomes (Eisenlohr-Moul et al., 2012), we also examined interactions between non-reactivity and non-judgment with observing thoughts, emotions, and sensations and present moment awareness. However, these results were insignificant and were dropped from our analytic model.

RESULTS

Table 1 presents means, standard deviations, and bivariate correlations among the primary study variables. Bivariate correlations indicated that women who reported greater sexual arousability toward their preferred sex showed greater tendency to put their experiences into words (describing) and overall trait mindfulness (full scale FFMQ).

Objective 1: Does Oxytocin Change in Response to the Sexual Arousal and Mindful Breathing Inductions? No and Yes

The unconditional model examined the rate of change in oxytocin across tasks. Contrary to our predictions, oxytocin did not significantly change in response to the sexual arousal induction ($p > 0.1$). However, there was a significant random effect [$\chi^2(62, N = 63) = 178.38$, $p < 0.001$], indicating that women varied in the extent to which oxytocin changed in response to the sexual arousal induction. In contrast to our objective that oxytocin would increase in response to mindful

TABLE 1 | Descriptive and correlations between study variables.

	Mean	*SD*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 Peak arousal toward preferred sex	6.13	2.08	–																	
2 Peak arousal toward non-preferred sex	4.65	2.32	0.34*	–																
3 Average of all peak arousal	4.86	1.72	0.77*	0.81*	–															
4 Observing	21.82	5.01	0.14	0.19	0.24	–														
5 Describing	13.2	3.38	0.28*	0.26*	0.29*	0.08	–													
6 Act with awareness	19.31	3.45	0.20	0.14	0.18	−0.16	0.21	–												
7 Non-judging	15.66	3.50	0.14	−0.03	0.10	−0.18	0.11	0.33*	–											
8 Non-reacting	12.64	3.30	0.03	−0.13	−0.06	0.19	−0.06	0.23	0.37*	–										
9 Full scale FFMQ	3.30	0.39	0.28*	0.18	0.31*	0.48*	0.48*	0.54*	0.54*	0.62*	–									
10 Mind wandering	3.74	0.96	0.04	0.19	0.13	0.02	0.05	0.16	−0.24	−0.22	−0.08	–								
11 Mindful relaxation	4.07	1.07	0.25*	0.24	0.27*	0.21	0.00	0.09	0.21	0.23	0.29*	0.04	–							
12 Oxytocin – baseline	11.20	7.00	−0.19	0.00	−0.08	0.02	−0.01	−0.30*	0.00	−0.07	−0.12	−0.27*	−0.05	–						
13 Oxytocin – arousal	11.74	6.43	−0.10	−0.03	−0.08	−0.05	−0.01	−0.28*	0.05	−0.12	−0.15	−0.20	0.03	0.85*	–					
14 Oxytocin – mindfulness	10.53	6.51	−0.10	0.02	−0.04	0.02	−0.03	−0.16	0.03	−0.13	−0.09	−0.20	−0.04	0.84*	0.89*	–				
15 Oxytocin – recovery	11.73	6.18	−0.30*	−0.12	−0.25	−0.10	0.00	−0.21	−0.08	−0.17	−0.21	−0.29*	−0.15	0.80*	0.82*	0.78*	–			
16 Cortisol – baseline	8.86	5.3	−0.02	−0.01	−0.08	−0.08	0.00	−0.04	−0.30*	−0.14	−0.21	−0.21	−0.09	0.25	0.17	0.17	0.23	–		
17 Cortisol – arousal	6.61	5.11	−0.08	−0.13	−0.13	−0.03	−0.14	−0.08	−0.23	−0.09	−0.21	−0.08	−0.11	0.21	0.11	0.14	0.15	0.62*	–	
18 Cortisol – mindfulness	6.38	4.64	−0.01	−0.02	−0.03	−0.11	−0.12	−0.02	−0.18	−0.16	−0.22	−0.05	−0.04	0.26*	0.27*	0.28*	0.23	0.55*	0.85*	–
19 Cortisol – recovery	6.41	5.13	0.03	0.03	0.00	−0.14	0.03	−0.03	−0.23	−0.14	−0.20	−0.02	0.03	0.20	0.28*	0.14	0.19	0.60*	0.67*	0.81*

*$p < 0.05$.

breathing, oxytocin significantly *decreased* in response to mindful breathing ($b = -0.13$, $SE = 0.05$, $p = 0.011$). We also found that change in oxytocin in response to mindful breathing varied significantly across women [$\chi^2(62, N = 63) = 110.93$, $p < 0.001$]. Following the mindfulness induction, during the recovery period, oxytocin increased, and surpassed baseline levels of oxytocin ($b = 0.12$, $SE = 0.05$, $p = 0.01$). The degree to which oxytocin increased during the recovery period varied significantly across women [$\chi^2(62, N = 63) = 105.07$, $p < 0.001$]. In summary, oxytocin did not significantly increase in response to the sexual arousal induction, decreased significantly in response to mindful breathing, and increased (passed baseline) during recovery.

Replication of Decreases in Oxytocin in Response to Mindful Breathing

Given the unexpected finding that mindful breathing *decreased* rather than increased oxytocin, we were concerned the nature of the arousal task may have influenced the direction of change in oxytocin in response to mindful breathing and whether this pattern was replicable. Thus, we examined the replicability of this pattern by examining the pattern of oxytocin in response to a laboratory stressor 2 weeks later. Similar to the first visit, oxytocin significantly decreased in response to the mindful breathing induction ($b = -0.14$, $SE = 0.04$, $p = 0.002$) and the rate of change varied significantly across women [$\chi^2(63, N = 62) = 97.16$, $p = 0.003$]. For those interested in the effects on stress, oxytocin increased in response to stress ($b = 0.12$, $SE = 0.04$, $p = 0.002$) and returned to baseline levels at recovery ($p > 0.2$). The visual pattern of oxytocin across the second visit (not shown) mirrored the pattern during the first visit; oxytocin increased during stress, decreased during mindful breathing, and returned to baseline during recovery.

Given that women significantly differed in how oxytocin changed across tasks, we next examined whether the facets of trait mindfulness and subjective responsiveness to the mindful breathing and sexual arousal inductions moderated the responsiveness of oxytocin – the rate of change in oxytocin across tasks (see **Figure 1** and **Table 2**). Given that sexual arousal has shown to differ across sexual orientation and relationship status (Lippa, 2006), we initially controlled for sexual orientation and relationship status. Consistent with standard practice, we dropped non-significant level 2 moderators (subjective sexual arousability for the non-preferred sex, observing thoughts, emotions, and sensation, describing internal experiences with words, non-reacting to internal experiences, relaxation responsiveness during mindful breathing, sexual orientation, and relationship status).

Objective 2: Do Women With Greater Trait Mindfulness Show Larger Changes in Oxytocin in Response to Sexual Arousal and Mindful Breathing? No and Yes

None of the facets of trait mindfulness moderated the change in oxytocin in response to the sexual arousal induction ($p > 0.2$).

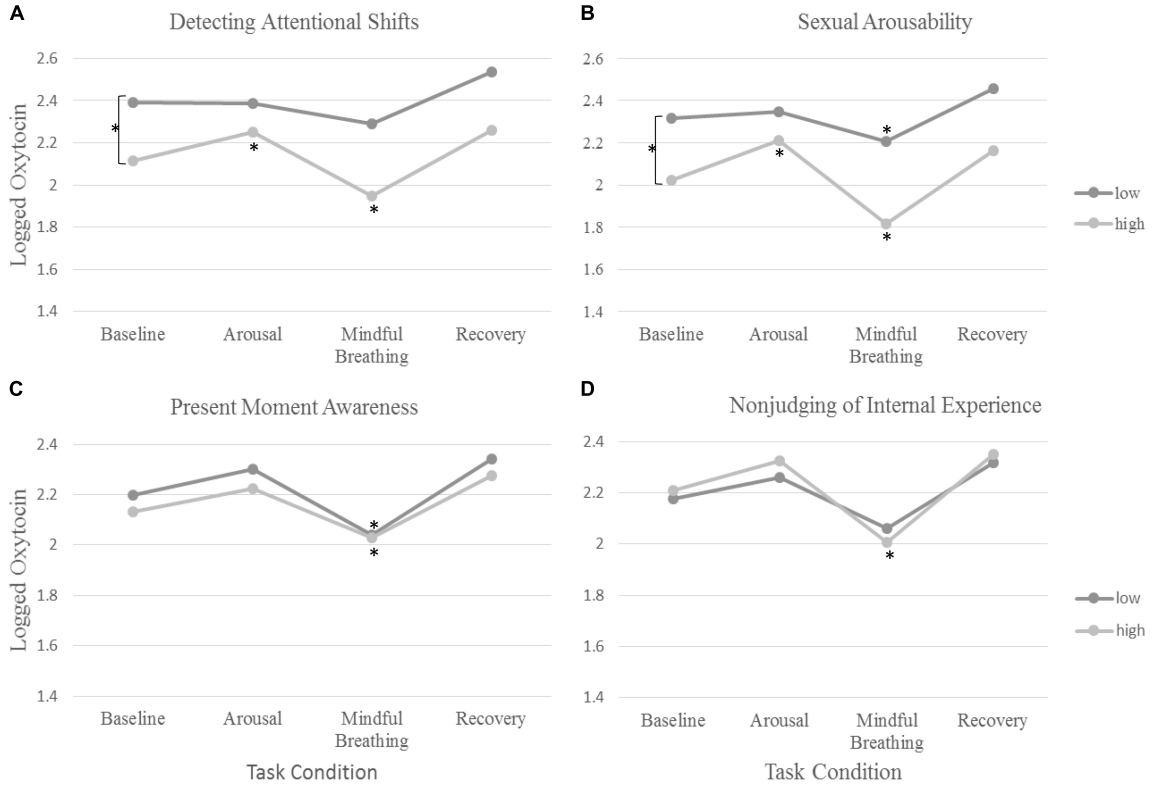

FIGURE 1 | The rate of change in oxytocin across tasks and differences across **(A)** detecting attentional shifts, **(B)** subjective sexual arousability, **(C)**, present-moment awareness, and **(D)** non-judging of internal experience. The symbol Asterisks (*) denote significance in change in oxytocin from baseline.

However, we found that two facets of trait mindfulness (Non-judging of internal experiences and acting with awareness) moderated the rate of change in oxytocin in response to mindful breathing. As expected, women who reported greater levels of Non-judging of Internal Experiences showed larger changes (decreases) in oxytocin in response to mindful breathing ($b = -0.02$, $SE = 0.01$, $p = 0.010$; see **Table 2**). Simple slopes revealed that women who reported lower levels Non-judging of Internal Experiences showed the least change in oxytocin in response to mindful breathing ($p > 0.06$).

Women who reported greater levels of Acting with Awareness showed weaker decreases in oxytocin in response to mindful breathing ($b = -0.07$, $SE = 0.03$, $p = 0.037$; see **Table 2**). Graphical analysis (see **Figure 1**) and simple slopes revealed that women who reported higher levels of acting with awareness showed lower levels of oxytocin at baseline ($b_{HighAware} = 2.24$, $SE_{HighAware} = 0.11$; $b_{LowAware} = 2.17$, $SE_{LowAware} = 0.12$; $\chi^2(2) = 411.61$, $p < 0.001$) but similar levels of oxytocin in response to mindful breathing as women who reported lower levels of Acting with Awareness ($b_{HighAware} = 2.07$, $SE_{HighAware} = 0.13$; $b_{LowAware} = 2.08$, $SE_{LowAware} = 0.12$). Hence, the weaker (but still significant) decreases among women with higher levels of Acting with Awareness, relative to those with lower levels of Acting with Awareness may have been a function of differences in baseline levels. However, these baseline differences evidenced in simple slopes tests were not significant in full model, which controls for but does not include oxytocin responses to mindful breathing (see **Table 2**).

In summary, women with higher levels of Non-judging of Internal Experiences showed larger changes in oxytocin in response to mindful breathing. Those with lower levels of Acting with Awareness showed larger changes in oxytocin in response to mindful breathing, but this effect may have been due to baseline differences rather than absolute levels of oxytocin during mindful breathing. Moreover, trait mindfulness was not related to change in oxytocin to the sexual arousal induction.

Objective 3A: Do Women Who Are Subjectively More Responsive to the Mindfulness Induction (or Sexual Arousal Induction) Show Larger Changes in Oxytocin in Response to the Mindfulness Induction (or Sexual Arousal Induction)? Yes

First, we examined whether women who are subjectively more responsive to the mindfulness induction show larger changes in oxytocin in response to the mindfulness induction. Women who felt more or less calm and relaxed during the mindful breathing induction did not show any differences in their rate of change in oxytocin. However, women who were

TABLE 2 | Multilevel model assessing subjective responses as moderators of the rate of change in oxytocin.

Fixed effect	Coefficient	Standard error	*t*-ratio	*p*-value
Baseline oxytocin, π_0				
Intercept, β_{00}	2.23	0.10	21.703	<0.001
Act with awareness, β_{01}	−0.03	0.03	−1.256	0.214
Non-judging of internal experience, β_{02}	0.01	0.03	0.253	0.801
Detecting attentional shifts, β_{03}	−0.28	0.09	−3.127	0.003
Peak sexual arousal, β_{04}	−0.11	0.05	−2.251	0.028
Rate of change in oxytocin to arousal, π_1				
Intercept, β_{10}	0.10	0.06	1.69	0.097
Act with awareness, β_{11}	0.00	0.01	−0.37	0.712
Non-judging of internal experience, β_{12}	0.01	0.01	0.665	0.509
Detecting attentional shifts, β_{13}	0.14	0.06	2.371	0.021
Peak sexual arousal, β_{14}	0.06	0.03	2.176	0.034
Rate of change in oxytocin to mindful breathing, π_2				
Intercept, β_{20}	−0.15	0.05	−3.188	0.002
Act with awareness, β_{21}	0.03	0.01	3.432	0.001
Non-judging of internal experience, β_{22}	−0.02	0.01	−2.651	0.01
Detecting attentional shifts, β_{23}	−0.07	0.03	−2.137	0.037
Peak sexual arousal, β_{24}	−0.04	0.02	−2.07	0.043
Rate of change in oxytocin after recovery, π_3				
Intercept, β_{30}	0.14	0.05	3.03	0.004
Cortisol, π_4				
Intercept, β_{40}	−0.03	0.02	−1.743	0.083

quicker to detect attentional shifts during mindful breathing moderated the rate of change in oxytocin. We found that women who were quicker to detect attentional shifts during mindful breathing had lower levels of oxytocin at baseline (b = −0.28, SE = 0.09, p = 0.003; see **Figure 1** and **Table 2**). Consistent with our hypotheses, we found that women who were quicker to detect attentional shifts during mindful breathing showed larger changes (decreases) in oxytocin in response to mindful breathing (b = −0.07, SE = 0.03, p = 0.037; see **Figure 1** and **Table 2**). Simple slopes revealed that only women were slow to detect when their mind wandered showed no change in their oxytocin in response to mindful breathing ($p > 0.06$).

Then, we examined whether women who are subjectively more responsive to the sexual arousal induction show larger changes in oxytocin in response to the sexual arousal induction. We found that women who were more arousable toward their preferred sex showed lower levels of oxytocin at baseline (b = −0.11, SE = 0.05, p = 0.028; see **Table 2**). Consistent with our hypotheses, we found that women who were more arousable showed larger changes in oxytocin in response to the sexual arousal induction (b = 0.06, SE = 0.03, p = 0.034; see **Table 2**). Simple slopes indicated that those with low or average levels of sexual arousability showed no significant change in oxytocin (see **Table 2**), whereas those who showed high levels of sexual arousability showed significant increases in oxytocin in response to the sexual arousal induction ($b_{HighArousability}$ = 0.19, $SE_{HighArousability}$ = 0.07; $\chi^2(1) = 7.07$, p = 0.008). In summary, subjective responsiveness to mindfulness was associated with change in oxytocin in response to mindful breathing and subjective arousability was associated with change in oxytocin in response to the sexual arousal induction.

Objective 3B: Is the Moderating Effect of Subjective Responsiveness on Change in Oxytocin Specific to the Type of Induction or Do Women Who Are More Subjectively Responsive Have an Oxytocinergic System That Is Broadly More Responsive to Various Contexts? the Latter

We examined whether women who are subjectively more responsive to the mindfulness induction show larger changes in oxytocin in response to the sexual arousal induction. Consistent with our hypothesis, women who were quicker to detect attentional shifts during mindful breathing showed larger changes in oxytocin during the sexual arousal induction (b = 0.14, SE = 0.06, p = 0.021; see **Figure 1** and **Table 2**). Simple slopes indicated that those with low or average levels of detecting attentional shifts showed no significant change in oxytocin during the sexual arousal induction (see **Table 2**). However, women who were quicker to detect attentional shifts during mindful breathing showed significant increases in oxytocin in response to the sexual arousal induction ($b_{HighMindful}$ = 0.14, $SE_{HighMindful}$ = 0.06; $\chi^2(1) = 5.75$, p = 0.016).

Next, we examined whether women who are subjectively more responsive to the sexual arousal induction show larger changes in oxytocin in response to the mindfulness induction. Consistent with this objective, women who were more subjectively arousable showed larger changes (decreases) in oxytocin in response to mindful breathing (b = −0.04, SE = 0.02, p = 0.043; see **Figure 1** and **Table 2**).

In summary, results of hypotheses 3A,B indicate that women who were more subjectively responsive to the sexual arousal induction and women who were more subjectively responsive to the mindful breathing induction showed larger changes in oxytocin, irrespective of the type of induction. Thus, we next examined whether women who are more responsive to the sexual arousal induction are also the same women who are more responsive to the mindfulness induction.

Objective 4: Are Women Who Are Subjectively and Biologically More Responsive to Mindful Breathing Also Subjectively and Biologically More Responsive to Erotic Stimuli? No and Yes

To investigate whether women who subjectively report greater responsiveness to the mindfulness induction also report greater responsiveness to the sexual arousal induction, we investigated

the bivariate correlations shown in **Table 1**. Women who were more arousable showed greater relaxation responses to the mindful breathing, but did not show any differences in their ability to detect attentional shifts (see **Table 1**).

We next assessed whether women who show larger changes in oxytocin in response to the sexual arousal induction also show larger changes in oxytocin in response to the mindful breathing induction. To obtain this correlation, we first outputted the residuals from the unconditional model, which represent the variation in the rate of change in oxytocin across individuals from the expected linear slope. We used linear regression to predict residuals of the rate of change in oxytocin to the mindful breathing induction from the residuals of the rate of change in oxytocin in response to the sexual arousal induction, controlling for residuals of oxytocin at baseline. In support of our hypothesis, change in oxytocin in response to the sexual arousal task, but not baseline oxytocin, predicted change in oxytocin in response to the mindful breathing induction ($b = -0.25$, $SE = 0.09$, $t(60) = -2.95$, $p = 0.005$). Specifically, women who showed larger increases in oxytocin in response to the sexual arousal induction showed larger decreases in oxytocin in response to mindful breathing.

In summary, women who were subjectively more arousable show greater relaxation responsiveness to mindful breathing, but relaxation responses to mindful breathing were not associated with change in oxytocin. In contrast, attentional responsiveness to mindful breathing (detecting attentional shifting) was associated with change in oxytocin to both the sexual arousal and mindful inductions, but was not associated with subjective arousability. Finally, women who showed greater changes in oxytocin in response to mindful breathing showed greater changes in oxytocin in response to the sexual arousal induction.

DISCUSSION

The current study sought to understand whether individual differences in responsiveness to mindful breathing were associated individual differences in responsiveness to a sexual arousal induction. We found that oxytocin significantly decreased during mindful breathing and increased during a recovery period, with no significant changes observed during the sexual arousal induction. Importantly, these main effects were heavily moderated. For example, women who were more attentive during mindful breathing and women who were more arousable during the sexual arousal induction were the only individuals who showed increases in oxytocin during the sexual arousal task. Such results make a significant contribution to our understanding of female sexual arousability by suggesting a potential role of oxytocin in linking mindfulness to sexual arousal.

We sought to understand whether some women reported a biological sensitivity to contextual influences, marked by a greater potential for mindfulness when engaging in mindful breathing and a greater potential for arousability during sexual contexts. On one hand, our results corroborate this objective by indicating that women who reported greater subjective arousability and subjective responsiveness to mindful breathing (detecting attentional shifts) reported larger changes in oxytocin in response to *both* mindful breathing and sexual arousal. This indicates that the moderating effects of subjective responsiveness on changes in oxytocin were not specific to the type of induction. Rather, women who subjectively respond more to mindful breathing and sexual arousal appear to have an oxytocinergic system that is generally more responsive (i.e., showed larger changes to both sexual and mindfulness contexts). Moreover, women who showed increases in oxytocin in response to the sexual arousal induction showed larger decreases in response to the mindful breathing induction. The culmination of these findings suggest that women who reported greater responsiveness to the arousal and mindful breathing inductions have an oxytocinergic system that is more responsive to *both* arousal and to mindfulness.

On the other hand, we found that the aspects of mindfulness that were associated with subjective arousability were not the same aspects of mindfulness that were associated with oxytocin responses to the sexual arousal induction. Specifically, women who reported greater subjective arousability showed greater relaxation responses during mindful breathing, but showed no differences in detecting attentional shifts, and the opposite was true for women with greater responsiveness in oxytocin. Thus, women who are more arousable may have an oxytocin system that is sensitive to both mindfulness and sexual arousal, but women who report greater arousability do not subjectively respond to the same aspects of mindfulness that are relevant to oxytocin. Relatedly, women with greater trait mindfulness (e.g., intentionally bringing present moment awareness to daily activities, observing without judging) showed larger changes in oxytocin in response to mindful breathing, but these facets were unrelated to subjective responses to the mindfulness induction and subjective and oxytocin responses to the sexual arousal induction. Although our results demonstrate links between oxytocin, mindfulness, and sexual arousability, the specific aspects of mindfulness practice relevant to oxytocin, trait mindfulness, and sexual arousability differ from one another. Perhaps such discrepancies represent important distinctions in the nuances of how mindfulness relates to subjective experiences and neuroendocrine release. However, we cannot rule out whether these results may have been due to reducing the number of items on the FFMQ or to the lack of assessing in-the-moment interoceptive awareness, which has been shown to mediate the link between mindfulness-based interventions for sexual interest and arousal disorders (see Arora and Brotto, 2017). Future research should use well-validated measures that assess various dimensions of mindfulness (Mehling et al., 2012; Gu et al., 2016) in order to examine the indirect pathways through which oxytocin impacts the link between various aspects of mindfulness (facets of trait mindfulness, attentional, and emotional responsiveness to mindfulness inductions) and sexual arousal.

Perhaps the most surprising finding from our study was that mindful breathing *decreased* oxytocin. Ancillary analysis indicated that this effect was replicated across two separate

time points, indicating that oxytocinergic decreases due to mindful breathing were not task or time specific, but marked reliable biological indicators of change due to mindful breathing. Decreases in oxytocin resulting from mindful breathing were further corroborated by results indicating that individuals who were better able to detect attentional shifts during mindful breathing not only showed greater decreases in oxytocin during mindful breathing, but also showed lower levels of baseline oxytocin. A similar, though more complicated, pattern emerged with trait mindfulness: those who were less judgmental of their internal experiences showed greater decreases in oxytocin in response to mindful breathing and those who acted with greater present-moment awareness showed lower levels of oxytocin across tasks but less – albeit still significant – decreases in oxytocin in response to mindful breathing. Whereas most of the research on oxytocin focuses on *increases* in oxytocin, the current data indicate that *reductions* in oxytocin may also be important for some tasks (i.e., mindful breathing).

Given the dearth of research on mindfulness and oxytocin and the potential benefits of oxytocinergic decreases, the exact meaning of the abovementioned effect remains unclear. However, Keeler et al. (2015) found that oxytocin increases or decreases depending on the degree of social attunement and responsive communication required in the task. Such findings speak to a differential effect of oxytocin depending the specifics of the task. Given that oxytocin plays a role in sexual arousal, orgasm, pair-bonding (Borrow and Cameron, 2012; Veening et al., 2015), social affiliation, emotional elevation (Piper et al., 2015; Pohling and Diessner, 2016), and stress (Taylor, 2006; Taylor et al., 2006; Chen et al., 2011; Kumsta and Heinrichs, 2012; Olff et al., 2013; Seltzer et al., 2014), it is possible that increases in oxytocin are necessary for stimulating and activating an individual to meet to the demands for sexual and social interactions. This is consistent with the presumed role of oxytocin to help organize an organisms response to the demands of a stressor or social situation by stimulating arousal and motivate an organism to seek out help and assistance from others when needed (Taylor, 2006; Kumsta and Heinrichs, 2012; Olff et al., 2013; Seltzer et al., 2014).

If increases in oxytocin are uniquely beneficial when the task requires some degree of activation, stimulation, or arousal (i.e., activation of the sympathetic system), our findings indicate that mindful breathing does not require the activation that increases in oxytocin provides, but rather is associated with decreases in oxytocin. Although mindfulness can stimulate general arousal (Ditto et al., 2006), the arousal processes involved in mindfulness practice appears to be more related to learning and memory, rather than mobilizing the arousal response to action, as indicated by theta wave activity and slowing of alpha wave activity (see Ivanovski and Malhi, 2007 for a review). Mindful breathing tends to rely more on a regulatory system thought to be mediated by the vagus nerve (Gao et al., 2016), responsible for physiological recovery, digestion, and rest. Considering mindfulness inductions as an enhanced recovery phase may explain why mindful breathing decreased oxytocin. Recent research indicates that oxytocin during stress was associated with faster vagal recovery, suggesting that oxytocin may actually boost recovery to stress more than it buffers reactivity (Engert et al., 2016). Hence, a decrease in oxytocin during mindful breathing might represent that effective recovery processes have been achieved. Given that sexual arousal induction activates similar processes as stress (e.g., both activate arousal), this may also explain why women who showed larger increases in oxytocin in response to the sexual arousal induction showed larger decreases in oxytocin in response to mindful breathing. That is, perhaps engagement of oxytocin during tasks that elicit arousal (sexual arousal in our study, stress in Engert et al., 2016) are associated with more effective recovery processes (i.e., larger decreases in oxytocin during mindful breathing in our study, faster vagal recovery in Engert et al., 2016).

The current study has important implications for the treatment of sexual arousal concerns. Mindfulness-based therapies have shown promise in their effectiveness in treating female arousal difficulties. Our study demonstrated that women with greater arousability also showed greater neuroendocrine responses to both arousal and mindfulness inductions. This findings begs the question, could such neuroendocrine responsivity, specifically oxytocin responsivity, differentiate who may benefit the most from mindfulness-based interventions? Prior research has reflected the question presented above, demonstrating that mindfulness-based therapies are more effective for women with histories childhood sexual abuse (Brotto et al., 2012). Given that women with histories of childhood sexual abuse show different stress responses (Meston and Lorenz, 2013) and oxytocinergic profiles (Heim et al., 2008; Pierrehumbert et al., 2010), perhaps such modifications in the oxytocinergic system makes these women more sensitive to interventions that impact their oxytocin system, such as mindfulness training.

Limitations and Future Directions

We argued that mindfulness leads to increased arousal, but our paradigm aimed to ensure assessment of sexual arousal that was not confounded from engaging in another task prior to the sexual arousal assessment. As such, all participants engaged in the sexual arousal task prior to engaging in the mindful breathing task. This paradigm also limited our ability to fully assess the process of recovery, which may have been particularly fruitful for understanding neuroendocrine correlates of mindfulness and sexual arousal. Future research would benefit from having some participants engage in an arousal task without engaging in mindful breathing (or another type of task), others engage in a mindful breathing task prior to the arousal task, as well as having other participants engage in mindful breathing task following the arousal task. Such a paradigm would allow for a more accurate understanding of *how* mindfulness practice is associated with augmentations in sexual arousal, as well as understanding the effect of mindfulness practice on recovery processes. Moreover, investigating how socially oriented forms of mindfulness practices differ from the more traditional focused breathing inductions may elucidate the complex pattern of results. Future research would benefit from larger sample sizes to obtain sufficient power to examine differences between women of various sexual orientation and interactions between the facets of trait mindfulness. Finally,

inclusion of measures related to compassion and general social functioning will be important considerations for future research. Understanding how compassion and general social functioning are involved in oxytocin, mindfulness, and arousal would provide important insights into our results and could have important clinical implications.

The fact that our results established a relation between subjective and neuroendocrine responsiveness to mindful breathing and to sexual arousability stimulates questions regarding the potential mediators and causal pathways. An interesting line of inquiry would be to investigate the role of self-compassion in mindfulness, oxytocin, and sexual arousal. Among women with sexual interest and desire disorders, change in overall sexual function through mindfulness-based interventions was mediated by self-compassion, although mindfulness based treatment did not actually increase self-compassion during treatment (Paterson et al., 2017). Hence, future research may consider comparing the pathways by which mindfulness, compassion, and oxytocin enhance arousal (does oxytocin promotes changes in mindfulness through compassion? does compassion facilitate change in oxytocin that results from mindfulness? etc.). Such understanding holds great potential in identifying why some women benefit more or less from mindfulness-based interventions. Another potential mediator not investigated in the current study is vagus nerve activity (Carter, 2017). During sexual arousal, oxytocin conveys sensory activity from the cervix, preparing the genitals for orgasm via the vagus nerve (Komisaruk and Sansone, 2003). Innervation by vagus nerve also enhances emotion regulation, is involved in mindfulness, and may be linked to compassion (Carter, 2017; Carter et al., 2017). Moreover, future investigation into the causal pathways between oxytocin, mindfulness, and arousability would benefit from investigating the effects of exogenous (intranasal) administration on sexual arousal and mindfulness. The precise pathways by which oxytocin may promote and result from *both* mindfulness and sexual arousal is a critical next step for future research.

CONCLUSION

In summary, the present research contributes to the growing number of studies indicating that mindfulness is associated with sexual arousal and provides an important first examination at the links between mindfulness, sexual arousal, and oxytocin. The current research contributed to our fundamental understanding of the role of attentional processes in sexual arousability, and the potential neuroendocrine underpinnings of these links. Specifically, results indicated that oxytocin plays a role in mindfulness, sexual arousal toward the preferred gender, and the link between the responsiveness to mindfulness and sexual arousability. Future research that continues to investigate both within-person and between-person variability in the neuroendocrine correlates of female sexual arousability will make important contributions to our evolving understanding of female sexuality.

ETHICS STATEMENT

The study was approved by University of Utah Institutional Review Board.

AUTHOR CONTRIBUTIONS

LD had full access to all the data in the study and took responsibility for the integrity of the data collection. JD and LD took responsibility for the accuracy of the data analysis. JD conducted the study design and data analysis. All authors contributed to the write up in efforts consistent with author order.

FUNDING

This research was supported by grants from the Gay and Lesbian Medical Association and the American Institute of Bisexuality, awarded to LD and a University of Utah departmental award granted to JD.

ACKNOWLEDGMENTS

We would like to thank Q Amber Porschatis for her assistance with data collection.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

CORRECTION
published: 18 October 2019
doi: 10.3389/fpsyg.2019.01737

Corrigendum: Subjective and Oxytocinergic Responses to Mindfulness Are Associated With Subjective and Oxytocinergic Responses to Sexual Arousal

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Keywords: oxytocin, sexual arousal, mindfulness, attentional shifts, women, sexual health

A Corrigendum on

Subjective and Oxytocinergic Responses to Mindfulness Are Associated With Subjective and Oxytocinergic Responses to Sexual Arousal

by Dickenson, J. A., Alley, J., and Diamond, L. M. (2019). Front. Psychol. 10:1101. doi: 10.3389/fpsyg.2019.01101

OPEN ACCESS

Edited and reviewed by:
Beate Ditzen, Heidelberg University Hospital, Germany

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Specialty section:
This article was submitted to Health Psychology, a section of the journal Frontiers in Psychology

Received: *22 May 2019*
Accepted: *12 July 2019*
Published: *18 October 2019*

Citation:
Dickenson JA, Alley J and Diamond LM (2019) Corrigendum: Subjective and Oxytocinergic Responses to Mindfulness Are Associated With Subjective and Oxytocinergic Responses to Sexual Arousal. Front. Psychol. 10:1737. doi: 10.3389/fpsyg.2019.01737

In the original article, there was an error. The citation for "Brotto et al., 2008" was incorrectly written. It should be "Brotto et al., 2012."

A correction has been made to the **Discussion**, paragraph seven:

"The current study has important implications for the treatment of sexual arousal concerns. Mindfulness-based therapies have shown promise in their effectiveness in treating female arousal difficulties. Our study demonstrated that women with greater arousability also showed greater neuroendocrine responses to both arousal and mindfulness inductions. This findings begs the question, could such neuroendocrine responsivity, specifically oxytocin responsivity, differentiate who may benefit the most from mindfulness-based interventions? Prior research has reflected the question presented above, demonstrating that mindfulness-based therapies are more effective for women with histories childhood sexual abuse (Brotto et al., 2012). Given that women with histories of childhood sexual abuse show different stress responses (Meston and Lorenz, 2013) and oxytocinergic profiles (Heim et al., 2008; Pierrehumbert et al., 2010), perhaps such modifications in the oxytocinergic system makes these women more sensitive to interventions that impact their oxytocin system, such as mindfulness training."

The reference has also been changed to reflect this correction from "Brotto, L. A., Basson, R., and Luria, M. (2008). A mindfulness-based group psychoeducational intervention targeting sexual arousal disorder in women. *J. Sex. Med.* 5, 1646–1659. doi: 10.1111/j.1743-6109.2008.00850.x" to "Brotto, L. A., Seal, B. N., and Rellini, A. (2012). Pilot study of a brief cognitive behavioral versus mindfulness-based intervention for women with sexual distress and a history of childhood sexual abuse. *J. Sex Marital Ther.* 38, 1–27. doi: 10.1080/0092623X.2011.569636".

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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